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(54) Title: NOVEL HUMAN CALCIUM SENSITIVE POTASSIUM CHANNEL SUBUNITS

(57) Abstract: The present invention is directed to novel human DNA sequences encoding calcium sensitive potassium channel subunits β2, β3a, β3b, β3c, and β3d, the proteins encoded by the DNA sequences, vectors comprising the DNA sequences, host cells containing the vectors, and methods of identifying inhibitors and agonists of calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunits and inhibitors and agonists of β3 gene transcription.

TITLE OF THE INVENTION

NOVEL HUMAN CALCIUM SENSITIVE POTASSIUM CHANNEL SUBUNITS

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

The present invention is directed to novel human DNA sequences encoding subunits of calcium sensitive potassium channels.

BACKGROUND OF THE INVENTION

Voltage-gated potassium channels form transmembrane pores that open or close in response to changes in cell membrane potential and selectively allow potassium ions to pass through the membrane. Voltage-gated potassium channels have been found in cells traditionally considered both excitable (*e.g.*, neurons, myocytes, secretory cells) and non-excitable (*e.g.*, T-cells, osteoclasts) and have been shown to maintain cell membrane potential and control the repolarization of action potentials in such cells. Following depolarization, voltage-gated potassium channels open, allowing potassium efflux and thus membrane repolarization. This behavior has made voltage-gated potassium channels important targets for drug discovery in connection with a variety of diseases. As a result, many voltage-gated potassium channels have been identified and many cloned. They are distinguishable by differences in primary structure and tissue-specific patterns of expression, as well as by electrophysiological and pharmacological properties. For reviews of voltage-gated potassium channels see Robertson, 1997, Trends Pharmacol. Sci. 18:474-483; Jan & Jan, 1997, J. Physiol. 505:267-282; Catterall, 1995, Ann. Rev. Biochem. 64:493-531.

Many functional voltage-gated potassium channels are believed to be tetramers of four α subunits, each of which contains six transmembrane spanning

segments. The αsubunits making up a tetramer may be the same (in the case of homotetramers) or may be different (in the case of heterotetramers). The membrane-spanning α subunits making up the tetramers may sometimes be associated with additional, β subunits, which may alter the behavior of the α subunits.

A particular type of voltage-gated potassium channel is the voltage-gated and calcium sensitive potassium channel, also known as the calcium sensitive potassium channel. Calcium sensitive potassium channels are present in a wide variety of cells and are unique among voltage-gated potassium channels because their activity is regulated not only by changes in membrane potential but also by intracellular calcium concentration. Plasma membrane depolarization and increases in cytoplasmic calcium concentration both raise the open probability of calcium sensitive potassium channels. Therefore, calcium sensitive potassium channels can serve as a link between cellular processes involving increases in intracellular calcium and membrane excitability. Calcium sensitive potassium channels are believed to play a negative feedback role by terminating signaling events involving an increase in intracellular calcium, *e.g.*, glucose mediated insulin release, blood vessel muscle tone, bronchial airway smooth muscle tone, and regulation of intraocular pressure. (Tanaka et al., 1997, J. Physiol. 502:545-557; Kaczorowski et al., 1996, J. Bioenerg. Biomem. 28:255-267; Vergara et al., 1998, Curr. Opin. Neurobiol. 8:321-329).

Certain calcium sensitive potassium channels have been isolated and studied. Functional calcium sensitive potassium channels are composed of α subunits that may be associated with smaller β subunits. The α subunit is believed to form the channel pore while a previously described β subunit increases the calcium sensitivity of the channel and makes the channel susceptible to regulation by certain substances, *e.g.*, dehydrosoyasaponin (McManus et al., 1995, Neuron 14:645-650). The calcium sensitive potassium channel from bovine tracheal smooth muscle was purified and shown to be composed of an ~130 kDa α subunit and a 31 kDa β subunit (Garcia-Calvo et al., 1994, J. Biol. Chem. 269:676-682). Tseng-Crank et al. (1994, Neuron 13:1315-1330) cloned nine related calcium sensitive potassium channel α subunits from human brain. These α subunits are thought to be splice variants derived from a single gene, the *h-slo* gene (Tseng-Crank et al., 1994, Neuron 13:1315-1330). Knauss et al., 1994, J. Biol. Chem. 269:17274-17278 purified and cloned a β subunit of a calcium sensitive potassium channel from tracheal smooth muscle.

In most cells, the opening of calcium sensitive potassium channels results in the generation of non-inactivating, hyperpolarizing potassium currents. However, in certain cells (*e.g.*, chromaffin cells of the adrenal gland and hippocampal neurons), the currents are inactivating. Following the discovery of the invention described herein, Wallner et al., 1999, Proc. Natl. Acad. Sci. USA 96:4137-4132 disclosed the existence of the human β2 calcium sensitive potassium channel subunit that, when combined with the α subunit, formed inactivating calcium sensitive potassium channels. The ability to confer inactivation was ascribed to the N-terminal 19 amino acids of the β2 subunit.

U.S. Patent No. 5,776,734 is directed to nucleic acids encoding the bovine and human β1 subunit of the calcium sensitive potassium channel. U.S. Patent No. 5,637,470 is directed to methods of identifying compounds that modulate the activity of calcium sensitive potassium channels.

SUMMARY OF THE INVENTION

The present invention is directed to novel human DNA sequences encoding β subunits of calcium sensitive potassium channels. The present invention includes DNAs that encode the β subunits β2, β3a, β3b, β3c, and β3d of human calcium sensitive potassium channels. The DNAs comprise the nucleotide sequences shown in SEQ.ID.NO.:1 (β2), SEQ.ID.NO.:3 (β3a), SEQ.ID.NO.:5 (β3b), SEQ.ID.NO.:7 (β3c), and SEQ.ID.NO.:9 (β3d). Also provided are proteins encoded by the novel DNA sequences. The proteins comprise the deduced amino acid sequences shown in SEQ.ID.NO.:2 (β2), SEQ.ID.NO.:4 (β3a), SEQ.ID.NO.:6 (β3b), SEQ.ID.NO.:8 (β3c), and SEQ.ID.NO.:10 (β3d). Methods of expressing the novel subunit proteins in recombinant systems are provided as well as methods of identifying activators and inhibitors of potassium channels comprising the subunits.

The present invention also includes a genomic DNA fragment containing the 5' portions of the β3a, β3b, β3c, and β3d subunits, as well as the 5' portion of the core portion of the β3 subunits. This genomic DNA fragment contains promoter elements for the subunits. Methods of screening for compounds which affect transcription of the gene encoding the β3a, β3b, β3c, and β3d subunits are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows a DNA sequence encoding the β2 subunit of the human calcium sensitive potassium channel (SEQ.ID.NO.:1). The start ATG codon is at position 271-273; the stop codon is at position 976-978. Figure 1B shows the deduced amino acid sequence (SEQ.ID.NO.:2) of the β2 subunit.

Figure 2A shows a DNA sequence encoding the β3a subunit of a human calcium sensitive potassium channel (SEQ.ID.NO.:3). The start ATG codon is at position 341-343; the stop codon is at position 1172-1174. Figure 2B shows the deduced amino acid sequence (SEQ.ID.NO.:4) of the β3a subunit.

Figure 3A shows a DNA sequence encoding the β3b subunit of a human calcium sensitive potassium channel (SEQ.ID.NO.:5). The start ATG codon is at position 796-798; the stop codon is at position 1567-1569. Figure 3B shows the deduced amino acid sequence (SEQ.ID.NO.:6) of the β3b subunit.

Figure 4A shows a DNA sequence encoding the β3c subunit of a human calcium sensitive potassium channel (SEQ.ID.NO.:7). The start ATG codon is at position 869-871; the stop codon is at position 1694-1696. Figure 4B shows the deduced amino acid sequence (SEQ.ID.NO.:8) of the β3c subunit.

Figure 5A shows a DNA sequence encoding the β3d subunit of a human and calcium sensitive potassium channel (SEQ.ID.NO.:9). The start ATG codon is at position 457-459; the stop codon is at position 1294-1296. Figure 5B shows the deduced amino acid sequence (SEQ.ID.NO.:10) of the β3d subunit.

Figure 6 shows an alignment of the deduced amino acid sequences of the human calcium sensitive potassium channel β1 (SEQ.ID.NO.:11), β2 (SEQ.ID.NO.:2), β3a (SEQ.ID.NO.:4), β3b (SEQ.ID.NO.:6), β3c (SEQ.ID.NO.:8), and β3d (SEQ.ID.NO.:10) subunits.

Figure 7 shows the effect of the co-expression of the novel β subunits of the present invention on the electrophysiological properties of the ion channel formed by the α subunit of a human calcium sensitive potassium channel. Figure 7A shows the current-voltage relations recorded in inside-out patches expressing calcium sensitive potassium channel α or α and β subunits. α and β subunit cRNAs were co-injected in 1:10 molar ratio (β in excess) to detect maximum effects. The voltage clamp protocol consisted of a pre-pulse to -160 mV (200 ms), followed by 20 mV depolarizing steps from -80 to +80 mV (500 ms); holding potential was -80 mV; internal Ca^{2+} was 30 μM. Subunits β3b and β3d did not induce noticeable changes in

the kinetics and voltage dependence of the channels formed by α subunits, although they might decrease current density. Figure 7B: Boltzmann equations were fit to normalized conductances for the records shown in 7A, which were calculated from peak currents and plotted as function of test potential. $V_{1/2}$ values are: 20 mV (α subunit alone); -55 mV (α +β2 subunit); 45.36 mV (α +β3a subunit); 20 mV (α + β3c subunit). Figure 7C shows that co-expression of β3 subunit RNAs in molar excess of α subunit RNAs (up to 10X) reduced, but did not eliminate, a non-inactivating component of calcium sensitive potassium channel current. Inactivation rates and fractional inactivating current were calculated as described in Example 2.

Figure 8A-N shows the genomic sequence of GenBank accession number AC007823.4 (SEQ.ID.NO.:20). The different splice variants of the β3 subunits are contained in nucleotides 1-40,467. The β3a-specific sequence is at positions 17,404-17,806; the β3b-specific sequence is at positions 24,710-25,507; the β3c/d sequence is at positions 32,590-33,514; the beginning of the β3 core sequence is at positions 33,515-33,705. The sequences involved in tissue specific expression (*e.g.*, promoters, enhancers, repressors) are likely to be located in nucleotides 1-17,404.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Whether a given human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein preparation is substantially free from other proteins can be determined by conventional techniques of assessing protein purity such as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

"Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other

nucleic acids. Thus, a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit nucleic acids. Whether a given human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit DNA preparation is substantially free from other nucleic acids can be determined by conventional techniques of assessing nucleic acid purity such as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, Northern or Southern blotting, or by sequencing.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

A polypeptide has "substantially the same biological activity as human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit" if that polypeptide is able to combine with a human calcium sensitive potassium channel α subunit thereby forming a functional potassium channel where the polypeptide confers upon the α subunit properties similar to those conferred by the β2, β3a, β3b, β3c, or β3d subunits and where the polypeptide has an amino acid sequence that is at least about 50% identical to SEQ.ID.NO.:2, 4, 6, 8, or 10 when measured by such standard programs as BLAST or FASTA. For example, a polypeptide that is 50% identical in amino acid sequence to β3a (SEQ.ID.NO.:4) and is able to confer upon the α subunit properties such that electrophysiological measurements of the ion channel formed by the polypeptide and the α subunit result in graphs such as those shown in Figure 7A-C for the β3a subunit and the α subunit is a polypeptide that has "substantially the same biological activity as human calcium sensitive potassium channel β3a subunit."

The present invention relates to the identification and cloning of DNAs encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d

subunits, components of human calcium sensitive potassium channels. Expressed sequence tags (ESTs) (GenBank accession numbers AA904191, AI299145 and AI301175) were identified by searching databases for sequences with homology to the β1 subunit. The cDNAs encoding the ESTs were purchased and sequenced in both directions. The clone encoding AA904191 was determined to encode the entire β2 subunit, since it contained in frame stop codons 5' to the start ATG of the open reading frame and the entire open reading frame.

The β2 coding sequence was then used to search the databases for additional β subunits. Contigs were assembled from the identified ESTs and used to search the database once again. Several ESTs were identified in this iterative manner (GenBank accession numbers AA195381, AA236930, AA236968, AA279911, AA761761 and AA934876). Available cDNAs encoding these ESTs were purchased and sequenced in both directions. None of these clones were full length. Because most were isolated in a preparation of tonsils enriched for B-cells, we performed 5' RACE (rapid amplification of cDNA ends) using gene-specific oligonucleotides in the 3' untranslated region (UTR) and commercially prepared cDNA from human spleen, another tissue rich in B-cells (Clontech catalog # 7412-1), as the template. Multiple DNA fragments were amplified in this manner, cloned and sequenced in both directions. Sequencing revealed 4 subfamilies of full length clones, differing only in their 5' ends: β3a, β3b, β3c, and β3d.

The human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunits of the present invention exhibit tissue specific patterns of expression. Northern blotting of mRNAs isolated from various tissues has shown that the β2 subunit is expressed predominately in uterus, heart, ovary, thyroid, fetal kidney, adrenal medulla, and pancreas; the β3a subunit is expressed predominately in heart and skeletal muscle; the β3b subunit is expressed in most tissues examined except for brain, skeletal muscle and testes. The β3c and/or β3d subunits have been found in pancreas.

The tissue specific expression patterns of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunits support the hypothesis that these different subunits may contribute to the functional diversity of calcium sensitive potassium channels observed in different tissues. Activators and inhibitors of specific calcium sensitive potassium channels containing specific subunits may, therefore, have pharmacological efficacy in different pathological conditions, depending on the

subunit composition of the calcium sensitive potassium channels involved in the specific pathological condition.

Chromosomal mapping studies have shown that both the β2 and β3 subunits map to human chromosome 3q23-ter. The β subunits of the present invention have about 30-45% amino acid sequence identity to the previously known human β1 subunit (GenBank accession no. U25138). The β2 and β3 subunits of the present invention have about 40% amino acid sequence identity to each other. The β3a, β3b, β3c, or β3d subunits differ only in their extreme N-terminal 1-20 amino acids, and are alternatively spliced variants of a single gene. Indeed, a genomic fragment of human DNA has been identified in the GenBank database that contains the 5' domains of β3a, β3b, β3c/d, and the beginning of the conserved core in a contiguous fragment (accession number AC007823.4). See Figure 8. Additionally, two bacterial artificial chromosomes (BACs) have been isolated which contain the conserved core domain. One of these BACs also contains β3c/d specific sequence. Therefore, we have identified overlapping BAC clones that together encode the entire β3 open reading frame. The β2 subunit is encoded by a separate gene.

The present invention provides DNAs encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits that are substantially free from other nucleic acids. The present invention also provides isolated and/or recombinant DNA molecules encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequences shown in SEQ.ID.NOs.:1, 3, 5, 7, or 9. cDNAs encoding each β3 subunit have been isolated exhibiting a sequence polymorphism, encoding either a serine or an asparagine at the amino acid position that is equivalent to position 143 of β3b. This represents amino acid 142 of the conserved core domain.

Accordingly, the present invention includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA encoding a polypeptide selected from the group consisting of: SEQ.ID.NO.:4; SEQ.ID.NO.:4 with an asparagine at position 163 instead of a serine; SEQ.ID.NO.:6; SEQ.ID.NO.:6 with a serine at position 143 instead of an asparagine; SEQ.ID.NO.:8; SEQ.ID.NO.:8 with an asparagine at position 161 instead of a serine; SEQ.ID.NO.:10; and SEQ.ID.NO.:10 with a serine at position 165 instead of an asparagine.

The present invention includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA encoding a polypeptide comprising the conserved β3 core amino acid sequence, positions 2-246 of SEQ.ID.NO.:6.

The present invention includes isolated DNA molecules as well as DNA molecules that are substantially free from other nucleic acids comprising the coding regions of SEQ.ID.NOs.:1, 3, 5, 7, and 9. Accordingly, the present invention includes isolated DNA molecules and DNA molecules substantially free from other nucleic acids having a sequence comprising positions 271 to 975 of SEQ.ID.NO.:1, positions 341 to 1171 of SEQ.ID.NO.:3, positions 796 to 1566 of SEQ.ID.NO.:5, positions 869 to 1693 of SEQ.ID.NO.:7, or positions 457 to 1293 of SEQ.ID.NO.:9.

Also included are recombinant DNA molecules having a nucleotide sequence comprising positions 271-975 of SEQ.ID.NO.:1, positions 341 to 1171 of SEQ.ID.NO.:3, positions 796 to 1566 of SEQ.ID.NO.:5, positions 869 to 1693 of SEQ.ID.NO.:7, or positions 457 to 1293 of SEQ.ID.NO.:9. The novel DNA sequences of the present invention encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits are not naturally linked, to form "recombinant DNA molecules" encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. Such other sequences can include DNA sequences that control transcription or translation such as, *e.g.*, translation initiation sequences, internal ribosome entry sites, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract, the FLAG epitope, the myc epitope, GST, or maltose binding protein. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

The present invention also includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA comprising genomic sequences of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. The present invention includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA comprising

SEQ.ID.NO.:20; positions 1-40,467 of SEQ.ID.NO.:20; positions 17,404-17,806 of SEQ.ID.NO.:20; positions 24,710-25,507 of SEQ.ID.NO.:20; positions 32,590-33,514 of SEQ.ID.NO.:20; positions 33,515-33,705 of SEQ.ID.NO.:20; or positions 1-17,404 of SEQ.ID.NO.:20.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NOs:1, 3, 5, 7, 9, or 20 under conditions of high stringency. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 5X SSC, 10X Denhardt's solution, 50% Formamide, 2% SDS and 100 μg/ml denatured salmon sperm DNA. Hybridization of 32P-labelled, random primed probe is carried out in 5X SSPE, 10X Denhardts solution, 50% Formamide, 2% SDS, 100ug/ml salmon sperm DNA at 42°C overnight. Washing of filters is done in 2X SSC, 0.05% SDS at 42°C for 40 minutes, followed by 0.1X SSC, 0.05% SDS at 65°C for 40 minutes.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequences of SEQ.ID.NOs:1, 3, 5, 7, or 9 but still encodes the same human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins as SEQ.ID.NOs:2, 4, 6, 8, or 10. Such synthetic DNAs are intended to be within the scope of the present invention.

Mutated forms of SEQ.ID.NOs:1, 3, 5, 7, or 9 are intended to be within the scope of the present invention. In particular, mutated forms of SEQ.ID.NOs:1, 3, 5, 7, or 9 which encode proteins that either do not interact with an α subunit or which when combined with α subunits give rise to calcium sensitive potassium channels having altered voltage dependence, calcium sensitivity, current kinetics (such as activation, inactivation or deactivation), or pharmacologic properties as compared to wild-type calcium sensitive potassium channels are within the scope of the present invention. Such mutant forms can differ from SEQ.ID.NOs:1, 3, 5, 7, or 9 by having nucleotide deletions, substitutions, or additions.

Also intended to be within the scope of the present invention are RNA molecules having sequences corresponding to SEQ.ID.NOs:1, 3, 5, 7, or 9. Antisense nucleotides, DNA or RNA, that are the reverse complements of SEQ.ID.NOs:1, 3, 5, 7, or 9, or portions thereof, are also within the scope of the present invention. In addition, polynucleotides based on SEQ.ID.NOs:1, 3, 5, 7, or 9 in which a small number of positions are substituted with non-natural or modified nucleotides such as inosine, methyl-cytosine, or deaza-guanosine are intended to be within the scope of the present invention. Polynucleotides of the present invention can also include sequences based on SEQ.ID.NOs:1, 3, 5, 7, or 9 but in which non-natural linkages between the nucleotides are present. Such non-natural linkages can be, *e.g.*, methylphosphonates, phosphorothioates, phosphorodithionates, phosphoroamidites, and phosphate esters. Polynucleotides of the present invention can also include sequences based on SEQ.ID.NOs:1, 3, 5, 7, or 9 but having de-phospho linkages as bridges between nucleotides, *e.g.*, siloxane, carbonate, carboxymethyl ester, acetamidate, carbamate, and thioether bridges. Other internucleotide linkages that can be present include N-vinyl, methacryloxyethyl, methacrylamide, or ethyleneimine linkages. Peptide nucleic acids based upon SEQ.ID.NOs:1, 3, 5, 7, or 9 are also included in the present invention.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such recombinant host cells can be cultured under suitable conditions to produce human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such recombinant host cells are also useful in the methods of identifying activators and inhibitors of calcium sensitive potassium channels described herein. An

expression vector containing DNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be used for the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, amphibian cells such as *Xenopus* oocytes, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cells and cell lines which are suitable for recombinant expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and which are widely available, include but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), CPAE (ATCC CCL 209), Saos-2 (ATCC HTB-85), ARPE-19 human retinal pigment epithelium (ATCC CRL-2302), *Xenopus* melanophores, and *Xenopus* oocytes.

A variety of mammalian expression vectors can be used to express recombinant human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pIZD35 (ATCC 37565), and pSV2-dhfr (ATCC 37146). Another suitable vector is the PT7TS oocyte expression vector.

Following expression in recombinant cells, human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be purified by conventional techniques to a level that is substantially free from other proteins. Techniques that can be used include ammonium sulfate precipitation, hydrophobic or hydrophilic interaction chromatography, ion exchange chromatography, affinity chromatography, phosphocellulose chromatography, size exclusion chromatography, preparative gel electrophoresis, and alcohol precipitation, .In some cases, it may be

advantageous to employ protein denaturing and/or refolding steps in addition to such techniques.

Certain voltage-gated potassium channel subunits have been found to require the expression of other voltage-gated potassium channel subunits in order to be properly expressed at high levels and inserted in membranes. For example, co-expression of KCNQ3 appears to enhance the expression of KCNQ2 in *Xenopus* oocytes (Wang et al., 1998, Science 282:1890-1893). Also, some voltage-gated potassium channel α subunits require other related αsubunits (Jegla and Salkoff, 1997, J. Neurosci. 17:32-44) or Kvβ2 subunits (Shi et al., 1995, Neuron 16:843-852). Accordingly, the recombinant expression of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins may, under certain circumstances, benefit from the co-expression of other proteins and such co-expression is intended to be within the scope of the present invention. A particularly preferred form of co-expression is the co-expression of a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein (or combinations thereof) with a human calcium sensitive potassium channel α subunit protein. Such co-expression can be effected by transfecting an expression vector encoding a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein into a cell that naturally expresses a human calcium sensitive potassium channel α subunit protein. Alternatively, an expression vector encoding a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein can be transfected into a cell in which an expression vector encoding a human calcium sensitive potassium channel α subunit protein has also been transfected. Preferably, such a cell does not naturally express human calcium sensitive potassium channel α or β subunits.

The present invention includes human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins substantially free from other proteins. The deduced amino acid sequences of the full-length human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins are shown in SEQ.ID.NOs.:2, 4, 6, 8, and 10, respectively. Thus, the present invention includes human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins substantially free from other proteins having the amino acid sequences SEQ.ID.NO.:2, SEQ.ID.NO.:4; SEQ.ID.NO.:4 with an asparagine at position 163 instead of a serine; SEQ.ID.NO.:6; SEQ.ID.NO.:6 with a serine at position 143 instead of an asparagine; SEQ.ID.NO.:8; SEQ.ID.NO.:8 with an asparagine at

position 161 instead of a serine; SEQ.ID.NO.:10; and SEQ.ID.NO.:10 with a serine at position 165 instead of an asparagine. The present invention also includes isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins having the amino acid sequences SEQ.ID.NO.:2, SEQ.ID.NO.:4; SEQ.ID.NO.:4 with an asparagine at position 163 instead of a serine; SEQ.ID.NO.:6; SEQ.ID.NO.:6 with a serine at position 143 instead of an asparagine; SEQ.ID.NO.:8; SEQ.ID.NO.:8 with an asparagine at position 161 instead of a serine; SEQ.ID.NO.:10; and SEQ.ID.NO.:10 with a serine at position 165 instead of an asparagine.

Mutated forms of human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins are intended to be within the scope of the present invention. In particular, mutated forms of SEQ.ID.NOs:2, 4, 6, 8, and 10 that give rise to calcium sensitive potassium channels having altered electrophysiological or pharmacological properties when combined with α subunits are within the scope of the present invention.

As with many proteins, it is possible to modify many of the amino acids of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and still retain substantially the same biological activity as for the original proteins. Thus, the present invention includes modified human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as naturally occurring human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NOs:2, 4, 6, 8, or 10 wherein the polypeptides still retain substantially the same biological activity as naturally occurring human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NOs:2, 4, 6, 8, or 10 wherein the polypeptides still retain substantially the same biological activity as naturally occurring human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. In particular, the present invention includes embodiments

where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in conserved positions. Conserved positions are those positions in which the human calcium sensitive potassium channel β1, β2, and any of the β3 subunits all have the same amino acid (see Figure 6).

The human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins of the present invention may contain post-translational modifications, *e.g.*, covalently linked carbohydrate, phosphorylation, myristoylation, palmyltoylation, *etc.*

The present invention also includes chimeric human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Chimeric human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins consist of a contiguous polypeptide sequence of at least a portion of a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein fused to a polypeptide sequence that is not from a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein.

The present invention also includes isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and DNA encoding these isolated subunits. Use of the term "isolated" indicates that the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein or DNA has been removed from its normal cellular environment. Thus, an isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein is the only protein present, but instead means that the isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein. Thus, a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein."

It is known that certain potassium channels subunits can interact to form heteromeric structures resulting in functional potassium channels. For example, KCNQ2 and KCNQ3 can assemble to form a heteromeric functional potassium channel (Wang et al., 1998, Science 282:1890-1893). Accordingly, it is believed likely that the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins of the present invention will also be able to form heteromeric structures with other proteins where such heteromeric structures constitute functional potassium channels. Thus, the present invention includes such heteromers comprising human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Preferred heteromers are those in which the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins of the present invention forms heteromers with calcium sensitive potassium channel α subunits.

DNA encoding the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be obtained by methods well known in the art. For example, a cDNA fragment encoding full-length human calcium sensitive potassium channel β2 subunit protein can be isolated from human uterus, ovary or pancreas cDNA by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the DNA sequence encoding the human calcium sensitive potassium channel β2 subunit protein shown in Figure 1A as SEQ.ID.NO.:1. Suitable primer pairs would be, *e.g.*:

5'-AAG ATG TTT ATA TGG ACC AGT GGC-3' (SEQ.ID.NO.:12)

and

5'-ACT CAT AAC AGA CTG CAC GTT AC-3' (SEQ.ID.NO.:13).

The above and subsequent primers are meant to be illustrative only; one skilled in the art would readily be able to design other suitable primers based upon SEQ.ID.NO.:1. Such primers could be produced by methods of oligonucleotide synthesis that are well known in the art.

In a similar manner, PCR primers can be selected and produced for the other human calcium sensitive potassium channel subunit proteins of the present invention. For example, for the human calcium sensitive potassium channel β3a subunit, suitable primer pairs would be, *e.g.*:

5'-GTC ATG CAG CCC TTC AGC ATC CC-3' (SEQ.ID.NO.:14)

and

5'-TTG CAG AAA TCA CAG ACA TCT GAA-3' (SEQ.ID.NO.:15).

A suitable cDNA template from which the human calcium sensitive potassium channel β3a subunit can be isolated is human heart, skeletal muscle or spleen cDNA.

For the human calcium sensitive potassium channel β3b subunit, suitable primer pairs would be, *e.g.*:

5'-GCA ATG ACA GCC TTT CCT GCC TC-3' (SEQ.ID.NO.:16)

and

5'-TTG CAG AAA TCA CAG ACA TCT GAA-3' (SEQ.ID.NO.:15).

A suitable cDNA template from which the human calcium sensitive potassium channel β3b subunit can be isolated is human spleen cDNA.

For the human calcium sensitive potassium channel β3c subunit, suitable primer pairs would be, *e.g.*:

5'-GAA ATG TTC CCC CTT CTT TAT GAG-3' (SEQ.ID.NO.:17)

and

5'-TTG CAG AAA TCA CAG ACA TCT GAA-3' (SEQ.ID.NO.:15).

A suitable cDNA template from which the human calcium sensitive potassium channel β3c subunit can be isolated is human pancreas or spleen cDNA.

For the human calcium sensitive potassium channel β3d subunit, suitable primer pairs would be, *e.g.*:

5'-GAG ATG GAC TTT TCA CCA AGC TCT-3' (SEQ.ID.NO.:18)

and

5'-TTG CAG AAA TCA CAG ACA TCT GAA-3' (SEQ.ID.NO.:15).

A suitable cDNA template from which the human calcium sensitive potassium channel β3d subunit can be isolated is human pancreas or spleen cDNA.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM $MgCl_2$, 200 μM for each dNTP, 50 mM KCl, 0.2 μM for each primer, 10 ng of DNA template, 0.05 units/μl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 25 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20

seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

Since the calcium sensitive potassium channel subunits of the present invention are highly homologous to one another, and to other potassium channel subunits, it is desirable to sequence the clones obtained by the herein-described methods, in order to verify that the desired calcium sensitive potassium channel β subunits have in fact been obtained.

By these methods, cDNA clones encoding the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be obtained. These cDNA clones can be cloned into suitable cloning vectors or expression vectors, *e.g.*, the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA). Human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can then be produced by transfecting expression vectors encoding the subunits or portions thereof into suitable host cells and growing the host cells under appropriate conditions. Human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can then be isolated by methods well known in the art.

As an alternative to the above-described PCR methods, cDNA clones encoding the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be isolated from cDNA libraries using as a probe oligonucleotides specific for each human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*, Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for particular human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits and that can be used to screen cDNA libraries can be readily designed based upon the DNA sequences shown in Figures 1-5 and can be synthesized by methods well-known in the art.

Genomic clones containing the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes can be obtained from commercially

available human PAC, YAC, or BAC libraries available from Research Genetics, Huntsville, AL. Alternatively, one may prepare genomic libraries, *e.g.*, in P1 artificial chromosome vectors, from which genomic clones containing the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes can be isolated, using probes based upon the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit DNA sequences disclosed herein. Methods of preparing such libraries are known in the art (see, *e.g.*, Ioannou *et al.*,1994, Nature Genet. 6:84-89).

The novel DNA sequences of the present invention can be used in various diagnostic methods. The present invention provides diagnostic methods for determining whether a patient carries a mutation in one or more of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes. In broad terms, such methods comprise determining the DNA sequence of a region in or near one or more of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes from the patient and comparing that sequence to the sequence from the corresponding region of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes from a non-affected person, *i.e.*, a person who does not have the condition which is being diagnosed, where a difference in sequence between the DNA sequence of the gene from the patient and the DNA sequence of the gene from the non-affected person indicates that the patient has a mutation in one or more of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes.

The present invention also provides oligonucleotide probes, based upon the sequences of SEQ.ID.NOs:1, 3, 5, 7, 9, or 20 that can be used in diagnostic methods to identify patients having mutated forms of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, to determine the level of expression of RNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, or to isolate genes homologous to human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. In particular, the present invention includes DNA oligonucleotides comprising at least about 10, 15, or 18 contiguous nucleotides of a sequence selected from the group consisting of: SEQ.ID.NOs:1, 3, 5, 7, 9, and 20 where the oligonucleotide probe comprises no stretch of contiguous nucleotides longer than 5 of a sequence selected from the group consisting of: SEQ.ID.NOs:1, 3, 5, 7, 9, and 20 other than the said at least about 10,

15, or 18 contiguous nucleotides. The oligonucleotides can be substantially free from other nucleic acids. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide can be packaged in kits for use as probes.

The present invention makes possible the recombinant expression of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in various cell types.

The β2, β3a, β3b, β3c, and β3d subunits of the human calcium sensitive potassium channel have been expressed in *Xenopus* oocytes, both by themselves and in combination with an α subunit of a large-conductance calcium-sensitive potassium channel (maxi-K channel). The β subunits do not express currents on their own. However, when co-expressed with the α subunit, the β2, β3a, and β3c subunits induce inactivation of calcium sensitive potassium currents (Figure 7). The rates of inactivation produced by β2, β3a and β3c are dependent upon voltage and internal calcium concentration; inactivation time constants reach a maximum at high depolarizations and high micromolar calcium for β2, β3a and β3c, τ_{inact} ~ 30-40 ms at 80 mV with 30 μM intracellular Ca^{2+}. Measurements of current-voltage dependence obtained in the presence of micromolar intracellular Ca^{2+} demonstrate that β2 subunits induce a large shift in the voltage dependence of activation (~80 mV towards negative potentials, with 30 μM Ca^{2+} in the bath; Figure 7B). This modulatory effect is similar to the one previously described for β1 subunits, which do not induce inactivation. (McManus et al., 1995, Neuron 14:645-650). In contrast, β3a, β3b, β3c, and β3d subunits do not shift the voltage dependence when compared to channels containing only α subunits (Figure 7B).

The present invention also makes possible the development of assays that measure the biological activity of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such assays using recombinantly expressed human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are especially of interest. Such assays can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such identified compounds can serve as "leads" for the development of pharmaceuticals that can be used to treat

patients having diseases in which it is beneficial to enhance or suppress calcium sensitive potassium channel activity.

In versions of the above-described assays, calcium sensitive potassium channels containing mutant human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are used and inhibitors or activators of the activity of the mutant calcium sensitive potassium channels are identified.

Preferred cell lines for recombinant expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are those which do not express endogenous potassium channels (*e.g.*, CV-1, NIH-3T3, CHO-K1, COS-7). Such cell lines can be loaded with ^{86}Rb, an ion which can pass through potassium channels. The ^{86}Rb-loaded cells can be exposed to collections of substances (*e.g.*, combinatorial libraries, natural products, analogues of lead compounds produced by medicinal chemistry) and those substances that are able to alter ^{86}Rb efflux identified. Such substances are likely to be activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

Activators and inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are likely to be substances that are capable of binding to calcium sensitive potassium channels. Accordingly, one type of assay determines whether one or more of a collection of substances is capable of such binding.

Accordingly, the present invention provides a method for identifying substances that bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing cells expressing a calcium sensitive potassium channel containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(b) exposing the cells containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins to a substance that is not known to bind calcium sensitive potassium channels;

(c) determining the amount of binding of the substance to the cells;

(d) comparing the amount of binding in step (c) to the amount of binding of the substance to control cells where the control cells are substantially

identical to the cells of step (a) except that the control cells do not express human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the amount of binding in step (c) is greater than the amount of binding of the substance to control cells, then the substance binds to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

Another version of this assay makes use of compounds that are known to bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. New binders are identified by virtue of their ability to potentiate, prevent, or displace the binding of the known compounds. Substances that have this ability are likely themselves to be inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

Accordingly, the present invention includes a method of identifying substances that bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and thus are likely to be inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing cells expressing calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(b) exposing the cells to a compound that is known to bind to the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) determining the amount of binding of the compound to the cells in the presence and in the absence of a substance not known to bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the amount of binding of the compound in the presence of the substance differs from that in the absence of the substance, then the substance binds calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and is likely to be an inhibitor or

activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

Generally, the known compound is labeled (*e.g.*, radioactively, enzymatically, fluorescently) in order to facilitate measuring its binding to the calcium sensitive potassium channels.

Once a substance has been identified by the above-described methods, it can be assayed in functional tests, such as those described herein, in order to determine whether it is an inhibitor or an activator.

In particular embodiments, the compound known to bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins is selected from the group consisting of: charybdotoxin, iberiotoxin, and dehydrosoyasaponin.

The present invention includes a method of identifying activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) recombinantly expressing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or mutant human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a host cell so that the recombinantly expressed human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins form calcium sensitive potassium channels by forming heteromers with other calcium sensitive potassium channel subunit proteins;

(b) measuring the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence and in the absence of a substance suspected of being an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where a change in the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

It may be advantageous to recombinantly express other subunits of calcium sensitive potassium channels such as, *e.g.*, an α subunit. Alternatively, it may be advantageous to use host cells that endogenously express such other subunits.

In particular embodiments, the biological activity is the production of a calcium sensitive potassium current, a FRET signal, or the efflux of ^{86}Rb.

In particular embodiments, a vector encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins is transferred into *Xenopus* oocytes in order to cause the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the oocytes. Alternatively, RNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be prepared *in vitro* and injected into the oocytes, also resulting in the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the oocytes. Following expression of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the oocytes, and following the formation of calcium sensitive potassium channels containing these subunits and other calcium sensitive potassium channel subunits (which other subunits may also be transferred into the oocytes), membrane currents are measured after the transmembrane voltage and/or internal calcium concentration is changed in steps. A change in membrane current is observed when the calcium sensitive potassium channels open or close, allowing or inhibiting potassium ion flow, respectively. Similar oocyte studies were reported for KCNQ2 and KCNQ3 potassium channels in Wang et al., 1998, Science 282:1890-1893 and this reference and references cited therein can be consulted for guidance as to how to carry out such studies.

Inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be identified by exposing the oocytes to collections of substances and determining whether the substances can block or diminish, or activate or enhance the membrane currents observed in the absence of the substance.

Accordingly, the present invention provides a method of identifying inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) expressing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a heterologous system such that calcium sensitive potassium channels containing the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are formed;

(b) changing the transmembrane potential or internal calcium concentration of the heterologous system in the presence and the absence of a substance suspected of being an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) measuring membrane potassium currents following step (b);

where if the membrane potassium currents measured in step (c) are greater in the absence rather than in the presence of the substance, then the substance is an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the membrane potassium currents measured in step (c) are less in the absence rather than in the presence of the substance, then the substance is an activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

In particular embodiments, the heterologous system is selected from the group consisting of: *Xenopus* oocytes and a mammalian cell line.

The present invention also includes assays for the identification of activators and inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins that are based upon fluorescence resonance energy transfer (FRET) between a first and a second fluorescent dye where the first dye is bound to one side of the plasma membrane of a cell expressing calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and the second dye is free to shuttle from one face of the membrane to the other face in response to changes in membrane potential. In certain embodiments, the first dye is impenetrable to the plasma membrane of the cells and is bound predominately to the extracellular surface of the plasma membrane. The second dye is trapped within the plasma membrane but is free to diffuse within the membrane. At normal (*i.e.*, negative) resting potentials of the membrane, the second dye is bound predominately to the inner surface of the extracellular face of the plasma membrane, thus placing the second dye in close proximity to the first dye. This close proximity allows for the generation of a large amount of FRET between the two dyes. Following membrane depolarization, the second dye moves from the extracellular face of the membrane to the intracellular face, thus increasing the distance between

the dyes. This increased distance results in a decrease in FRET, with a corresponding increase in fluorescent emission derived from the first dye and a corresponding decrease in the fluorescent emission from the second dye. In this way, the amount of FRET between the two dyes can be used to measure the polarization state of the membrane. For a fuller description of this technique, see González & Tsien, 1997, Chemistry & Biology 4:269-277. See also González & Tsien, 1995, Biophys. J. 69:1272-1280 and U.S. Patent No. 5,661,035.

In certain embodiments, the first dye is a fluorescent lectin or a fluorescent phospholipid that acts as the fluorescent donor. Examples of such a first dye are: a coumarin-labeled phosphatidylethanolamine (*e.g.*, N-(6-chloro-7-hydroxy-2-oxo-2H--1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidyl-ethanolamine) or N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoylphosphatidylethanolamine); a fluorescently-labeled lectin (*e.g.*, fluorescein-labeled wheat germ agglutinin). In certain embodiments, the second dye is an oxonol that acts as the fluorescent acceptor. Examples of such a second dye are: bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols (*e.g.*, bis(1,3-dihexyl-2-thiobarbiturate)trimethineoxonol) or pentamethineoxonol analogues (*e.g.*, bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; or bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol). See González & Tsien, 1997, Chemistry & Biology 4:269-277 for methods of synthesizing various dyes suitable for use in the present invention. In certain embodiments, the assay may comprise a natural carotenoid, *e.g.*, astaxanthin, in order to reduce photodynamic damage due to singlet oxygen.

The above described assays can be utilized to discover activators and inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such assays will generally utilize cells that express calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, *e.g.*, by transfection with expression vectors encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and, optionally, other calcium sensitive potassium channel subunits. In such cells, depolarization of the membrane potential as well as increases in intracellular calcium concentration will tend to open the calcium sensitive potassium channels. This will result in potassium efflux, tending to counteract the depolarization. In other words,

the cells will tend to repolarize. The presence of an inhibitor of the calcium sensitive potassium channel will prevent, or diminish, this repolarization. Thus, membrane potential will tend to become more positive (*i.e.*, depolarized) in the presence of inhibitors. Activators of the calcium sensitive potassium channel will open this channel and thus tend to hyperpolarize the membrane potential. Changes in membrane potential (depolarizations and hyperpolarizations) that are caused by inhibitors and activators of calcium sensitive potassium channels can be monitored by the assays using FRET described above.

Accordingly, the present invention provides a method of identifying activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing test cells comprising:

(1) an expression vector that directs the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the cells so that calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunit proteins are formed in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane of the cells; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane of the cells to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is greater than the amount of FRET exhibited by the control cells, the substance is an activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d,-subunit proteins;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

The present invention also provides a method of identifying inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing test cells comprising:

(1) an expression vector that directs the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the cells so that calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunit proteins are formed in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane of the cells; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane of the cells to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is less than the amount of FRET exhibited by the control cells, the substance is an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

In a variation of the assay described above, instead of the cell's membrane potential being allowed to reach steady state on its own, the membrane potential is artificially set at a potential in which the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are open. This can be done, *e.g.*, by variation of the external K+ concentration in a known manner (*e.g.*, increased concentrations of external K+). If such cells, having open calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, are exposed to inhibitors, the calcium sensitive potassium channels will be blocked, and the cells' membrane potentials will be depolarized. This depolarization can be observed as a decrease in FRET.

In a variation of the assay described above, instead of the cell's membrane potential being allowed to reach steady state on its own, the membrane potential is artificially set at a potential in which the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are open by coexpression of another depolarizing current. If such cells, having open calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, are exposed to inhibitors, the calcium sensitive potassium channels will be blocked, and the cells' membrane potentials will be depolarized. This depolarization can be observed as a decrease in FRET. If such cells, having open calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, are exposed to activators, the balance of the calcium sensitive potassium current and the additional depolarizing current will shift (*i.e.*, the calcium sensitive potassium current will make a larger contribution to the total current) and the cell's membrane potential will be hyperpolarized. This polarization may be observed as an increase in FRET.

Accordingly, the present invention provides a method of identifying inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing cells comprising:

(1) an expression vector that directs the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit

proteins in the cells so that calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunit proteins are formed in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane of the cells; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane of the cells to the other face in response to changes in membrane potential;

(b) adjusting the membrane potential of the cells such that the ion channel formed by the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins is open;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells;

(d) repeating step (b) and step (c) while the cells are exposed to a substance that is suspected of being an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the amount of FRET exhibited by the cells that are exposed to the substance is different than the amount of FRET exhibited by the cells that have not been exposed to the substance, then the substance is an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

In particular embodiments of the above-described methods, the cells contain an expression vector encoding a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein. In particular embodiments of the above-described methods, the expression vector is transfected into the test cells.

In particular embodiments of the above-described methods, the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein has an amino acid sequence selected from the group consisting of: SEQ.ID.NO.:2, 4, 6, 8, and 10.

In particular embodiments of the above-described methods, the first fluorescent dye is selected from the group consisting of: a fluorescent lectin; a fluorescent phospholipid; a coumarin-labeled phosphatidylethanolamine; N-(6-chloro-7-hydroxy-2-oxo-2H--1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidyl-

ethanolamine); N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoyl-phosphatidylethanolamine); and fluorescein-labeled wheat germ agglutinin.

In particular embodiments of the above-described methods, the second fluorescent dye is selected from the group consisting of: an oxonol that acts as the fluorescent acceptor; bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)trimethineoxonol; bis(1,3-dialkyl-2-thiobarbiturate) quatramethineoxonols; bis(1,3-dialkyl-2-thiobarbiturate)pentamethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol); and bis(1,3-dialkyl-2-thiobarbiturate) hexamethineoxonols.

In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), *Xenopus* melanophores, or *Xenopus* oocytes.

In particular embodiments of the above-described methods, the control cells do not comprise item (a)(1) but do comprise items (a)(2) and (a)(3).

In assays to identify activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, it may be advantageous to co-express another calcium sensitive potassium channel subunit besides the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit. In particular, it may be advantageous to co-express a calcium sensitive potassium channel α subunit. Preferably, this is done by co-transfecting into the cells an expression vector encoding the other subunit. Suitable other subunits are, *e.g.*, the human calcium sensitive potassium channel α subunit *h-slo* (GenBank accession no. U11058), the mouse calcium sensitive potassium channel α subunit *m-slo* (GenBank accession no. U09383), the small conductance calcium sensitive potassium α subunits (GenBank accession nos. U69883, U69882, AF031815), or the intermediate conductance calcium sensitive potassium channel α subunit (GenBank accession no. AF022797).

Small regions of genomic sequences in proximity to a gene often regulate the transcription of that gene. These sequences are referred to as cis-acting elements. The proteins that bind these DNA sequences and directly affect the ability of the transcriptional machinery to bind or transcribe the gene are referred to as trans-acting elements. The cis-acting transcriptional regulatory elements are most often 5' of the transcription start site, but have been located within and 3' of the transcribed portion of genes as well. Depending on their effects on the rate of transcription, these sequences can be divided into three categories: promoters, enhancers, and repressors. A promoter independently allows transcription of the gene, while an enhancer increases the rate of transcription but is not capable of inducing transcription independently of the promoter. A repressor element inhibits transcription directed by a promoter element. Methods for identifying these elements are well know in the field and are described in Ausubel et al., eds., 1989, Current Protocols in Molecular Biology, sections 9.6-9.8, and 12.0-12.11, John Wiley & Sons, New York, NY.

Accordingly, the novel genomic sequences (SEQ.ID.NO.:20, Figure 8) and isolated BAC clones of the present invention make possible methods for identifying 1) DNA sequences required for transcriptional control of gene expression, 2) proteins involved in transcriptional regulation and 3) compounds which modulate the rate of transcription of the β3 gene. Such assays utilize isolated and/or recombinant DNA comprising portions of SEQ.ID.NO.:20, positions 1 to 17,436, inserted into vectors upstream of the open reading frame of a reporter protein.

Useful reporter proteins are ones that are not expressed in the cells to be assayed (or are easily distinguished from endogenous proteins), have a linear relationship between the abundance of the transcript and the abundance of the reporter protein, and have a large window between the minimum detection level and saturation of detection system. Ideally, the abundance of the reporter protein is quickly measured by an enzymatic reaction, fluorescence detection, immunoassay or other means. Typical reporter proteins include, but are not limited to, the following: Chloramphenicol Acetyltransferase (CAT), firefly luciferase, Beta-Lactamase, Beta-Galactosidase, Secreted Alkaline Phosphatase (SEAP), human Growth Hormone (hGH), Green Fluorescent Protein (GFP) and GFP derivatives. Reporter vectors incorporating these proteins are commercially available, as are similar reporter vectors containing constitutive promoters, enhancers, or both (Clontech).

The present invention provides a method for identifying nucleotide sequences involved in transcriptional regulation of β3 gene expression. Once a fragment of at least 6 contiguous nucleotides of DNA from SEQ.ID.NO.:20, positions 1-17,436, has been inserted upstream of the reporter cDNA in a promoter-reporter vector, the vector is then transfected into cells that either do or do not endogenously express one or more of the calcium sensitive potassium channel subunits β3a, β3b, β3c or β3d. Promoter-reporter vectors may contain promoters, enhancers, both, or neither. Transfected cells are then assayed for the amount of reporter protein present. Because both transfection efficiency and transcription rate directly affect reporter protein level, it is useful in these assays to determine the transfection efficiency by co-transfecting a second vector (molar ratio 1:1) containing a distinct reporter behind a constitutive promoter, and determining the fraction of transfected cells.

In versions of the above assay, vectors are constructed with fragments of SEQ.ID.NO.:20 inserted upstream of a reporter cDNA with no other enhancer or promoter elements. These vectors (with and without fragments of SEQ.ID.NO.:20) are transfected into cells that endogenously express β3 subunits. Calcium sensitive potassium channel β3 subunit promoter elements are identified by the ability of these 5' gene fragments to stimulate reporter expression above the levels observed in the parent vector. The minimum required promoter sequence is then identified by successively deleting regions of the identified promoter fragment, and repeating the assay.

Another version of the assay incorporates fragments of SEQ.ID.NO.:20 inserted upstream of the reporter cDNA in a promoter-reporter vector containing an enhancer element. These vectors (with and without fragments of SEQ.ID.NO.:20) are transfected into cells that endogenously express β3 subunits. Weak calcium sensitive potassium channel β3 subunit promoter elements are identified by the ability of these 5' gene fragments to stimulate reporter expression above the levels observed in the parent vector. The minimum required weak promoter sequence can then be identified by successively deleting regions of the identified weak promoter fragment and repeating the assay

A different version of the assay incorporates fragments of SEQ.ID.NO.:20 inserted upstream of the reporter cDNA in a promoter-reporter vector with a constitutive promoter upstream. These vectors (with and without fragments of SEQ.ID.NO.:20) are transfected into cells that do not endogenously express β3

subunits. Calcium sensitive potassium channel β3 subunit repressor elements are identified by the ability of these 5' gene fragments to prevent or reduce reporter expression below the levels observed in the parent vector. The minimum required repressor sequence is then identified by successively deleting regions of the identified repressor fragment and repeating the assay.

In view of the above, the present invention provides a method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector;

(c) comparing the abundance of the reporter protein in the cells of step (b) to the abundance of the reporter protein in cells transfected with the vector without fragments of the promoter region of the β3 gene;

where fragments of the promoter region of the β3 gene which increase the abundance of the reporter protein in the absence of other promoter elements only in cells which endogenously express β3a, β3b, β3c, or β3d subunits are promoter elements; sequences which decrease the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which do not endogenously express β3a, β3b, β3c, or β3d subunits are repressor elements; and sequences which increase the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which endogenously express β3a, β3b, β3c, or β3d subunits are enhancer elements.

In particular embodiments, the vector contains promoter or enhancer sequence elements which function independently of the fragments of the promoter region of the β3 gene.

In particular embodiments, the abundance of the reporter protein is normalized with respect to the fraction of transfected cells.

The binding of nuclear proteins to these sequences can be confirmed by gel-shift assays. A radiolabeled DNA fragment corresponding to the minimal sequence required to affect transcription is incubated with nuclear protein extracts from cells used to identify the regulatory DNA element, or tissues endogenously

expressing β3 subunits. If a protein factor binds that sequence, the mobility in a gel will be altered, resulting in an apparent shift in the size of the radiolabeled fragment.

Transcription factors often are able to recognize more than one specific nucleotide sequence. As such, variations of sequences identified as minimal promoters, enhancers or repressors necessary for transcriptional regulation of the β3 gene in SEQ.ID.NO.:20, positions 1-17,436, which retain the ability to influence transcription as detected in the above described assays are intended to be included in the present invention.

Minimal promoter, enhancer or repressor DNA fragments thus identified can then be used to identify and/or isolate proteins that influence transcriptional activity of the β3 gene. Several methods are well known in the field, some of which are described in Ausubel et al., eds., 1989, Current Protocols in Molecular Biology, sections 12.0-12.11, John Wiley & Sons, New York, NY.

In one method, gel shift assays described above can be performed with cloned or purified known transcription factors, to determine if they are capable of binding sequences involved in transcriptional regulation. Alternatively, super-shift assays can be performed in which an antibody that recognizes a particular transcription factor is added to the transcription factor-DNA complex. If the antibody binds to the transcription factor, which in turn binds the radiolabeled DNA fragment, the mobility of the complex in a gel is further altered, resulting in a super-shift compared to the DNA alone. Using antibodies that recognize a specific transcription factor, or a class of transcription factors, allows identification of the factors involved in β3 gene regulation. Variations of sequences identified as minimal promoters, enhancers or repressors necessary for transcriptional regulation of the β3 gene in SEQ.ID.NO.:20, positions 1-17,436, which retain the ability to undergo gel shifts or super-shifts as described in the above assays are intended to be included in the present invention.

In view of the above, the present invention provides a method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells and separating the incubation on a gel;

where fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene that migrate differently in a gel ('undergo a shift') after incubation with nuclear extracts from cells are DNA sequences which bind nuclear factors which promote, enhance or repress β3 gene expression.

In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the method of claim 18.

In particular embodiments, the cells express β3a, β3b, β3c, or β3d subunits.

In particular embodiments, the cells do not express β3a, β3b, β3c, or β3d subunits.

The present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with cloned or purified transcription factors and separating the incubation on a gel;

where factors which bind β3 gene promoter sequence elements will induce a shift in the migration of the radiolabeled DNA fragments, and are involved in β3 gene transcription regulation.

In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

The present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells and separating the incubation on a gel;

(b) adding an antibody that specifically recognizes a single transcription factor or a family of transcription factors to the incubation of step (a), followed by separating the incubation on a gel;

where a super-shift in mobility of the double stranded DNA in step (b) as compared to step (a) indicates that a transcription factor recognized by the antibody binds the double stranded DNA.

In another method, the transcription factors that bind SEQ.ID.NO.:20, positions 1-17,436, and regulate transcription can be purified. DNA fragments corresponding to the minimal sequence required to affect transcription are covalently linked to a matrix (typically an agarose bead). This matrix is then incubated with nuclear extracts of cells that contain factors which bind the minimal element. The matrix is then washed free of non-specific proteins and the factor(s) are eluted with an excess of the DNA element, or by denaturation. Purified proteins can then be identified by immunoassay, protein sequencing, or other means.

Accordingly, the present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating nuclear extracts from cells with the matrix;

(c) washing non-binding proteins from the nuclear extract from the matrix;

(d) eluting bound proteins from the matrix with excess double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

where the eluted proteins from step (d) are nuclear factors involved in β3 gene transcription regulation.

In particular embodiments, the method further comprises separating the eluted proteins from step (d) on a gel and staining the gel to test for purity of the eluted proteins.

In particular embodiments, the method further comprises sequencing the proteins that have been separated on the gel.

In particular embodiments, the method further comprises immunological analysis of the proteins that have been separated on the gel with antibodies directed towards known transcription factors to identify the eluted proteins by western blot or immunoprecipitation.

In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

In a different approach, cDNAs encoding the transcription factors that bind SEQ.ID.NO.:20; positions 1-17,436 can be cloned by several methods. In one version, the minimal DNA sequence is radiolabeled and used to screen an expression library made from tissues or cell lines that endogenously express the β3 gene. Phage containing cDNA encoding the transcription factor are induced to express fusion proteins that target the transcription factor to its surface. Such phage plaques are identified by their ability to bind radiolabeled DNA sequences containing the minimal DNA sequence.

Accordingly, the present invention provides a method of identifying clones encoding nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) screening an expression library with radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436)

(b) determining which clones of the library bind the radiolabeled fragments of double stranded DNA;

(c) amplifying and sequencing the clones of step (b).

In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

Another cloning approach involves phage expressing transcription factor fusion proteins at their surface. In this approach, the minimal DNA sequence is linked to a matrix. A phage expression library is then passed over the matrix and washed. Only phage containing the transcription factor bind the matrix. Bound phage are eluted with excess minimal DNA sequence and purified. cDNA encoding the transcription factor is then isolated from the phage and sequenced.

Accordingly, the present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating phage expressing cDNA encoded fusion proteins at their surface with the matrix;

(c) removing phage that do not bind to the matrix by washing;

(d) eluting phage bound to the matrix with excess fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

where the phage eluted in step (d) encode nuclear factors involved in β3 gene transcription regulation.

In particular embodiments, the DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

In particular embodiments, the phage eluted at step (d) are amplified and sequenced.

A separate transcription factor cloning approach is the yeast 'one-hybrid' method (available in kit form from Clontech). In this method, yeast strains are made that contain several copies (three suggested) of the minimal element upstream of a reporter. A cDNA library is made such that each vector contains a cDNA that will be expressed as a fusion protein with the transcription activation domain of a yeast promoter. Thus, any fusion protein that specifically binds the DNA of interest will induce expression of the reporter protein. The vector containing the cDNA is then isolated from the yeast and sequenced.

Accordingly, the present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) constructing a yeast strain that contains a few to several copies of a fragment of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) preceding a cDNA encoding a reporter protein;

(b) constructing a cDNA library from cells in a vector that allows formation of fusion proteins encoded by the inserted cDNA and a transcription activation domain;

(c) transforming the library of (b) into the yeast strain of (a) and isolating colonies of yeast displaying expression of the reporter protein.

In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

In particular embodiments, the method further comprises purifying the vectors from the isolated colonies and sequencing the cDNA in the vectors.

Since transcription factors often are able to recognize more than one specific nucleotide sequence, variations of sequences identified as minimal promoters, enhancers or repressors necessary for transcriptional regulation of the β3 gene in SEQ.ID.NO.:20; positions 1-17,436, that can be bound by transcription factors as detected in the above described assays are intended to be included in the present invention.

Identification of nucleotide sequences involved in transcriptional regulation of β3 gene expression by the methods described above allows for the development of assays that can be used to screen collections of substances to identify those substances that enhance or inhibit transcription of the β3 gene. Fragments of the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) that have been shown to be involved in transcriptional regulation are linked to the coding sequence of a reporter gene in a suitable vector and are then transferred to appropriate cells. The abundance of the reporter protein in the cells is determined. The cells are then exposed to compounds that are suspected of being capable of enhancing or inhibiting the rate of transcription of the β3 gene. If the compound actually is capable of enhancing the rate of transcription of the β3 gene, then the abundance of the reporter protein will be increased when the cells are exposed to the compound. Conversely, if the compound actually is capable of inhibiting the rate of transcription of the β3 gene, then the abundance of the reporter protein will be decreased when the cells are exposed to the compound.

Accordingly, the present invention provides a method of identifying substances that enhance or inhibit the rate of transcription of the β3 gene comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector in the presence and absence of a compound;

where (1) if the presence of the compound decreases the abundance of the reporter protein, then the compound is a substance that inhibits the rate of transcription of the β3 gene; (2) if the presence of the compound increases the

abundance of the reporter protein, then the compound is a substance that enhances the rate of transcription of the β3 gene.

In particular embodiments, the method further comprises a control in which the effect of the compound on the abundance of the reporter protein in control cells is measured, where the control cells are cells that are essentially the same as the cells of step (b) except that the control cells have been transfected with a vector that lacks fragments of the promoter region of the β3 gene.

While the above-described methods are explicitly directed to testing whether "a" substance is an activator or inhibitor of the transcription the β3 gene or the function of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, *e.g.*, combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Accordingly, the use of collections of substances, or individual members of such collections, as the substance in the above-described methods is within the scope of the present invention. In particular, it is envisioned that libraries that have been designed to incorporate chemical structures that are known to be associated with potassium ion channel modulation, *e.g.*, dihydrobenzopyran libraries for potassium channel activators (International Patent Publication WO 95/30642) or biphenyl-derivative libraries for potassium channel inhibitors (International Patent Publication WO 95/04277) will be especially suitable.

The present invention includes pharmaceutical compositions comprising activators or inhibitors of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins that have been identified by the herein-described methods as well as activators or inhibitors of β3 gene transcription. The activators or inhibitors are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing activators or inhibitors and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the activators or inhibitors.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, gender, and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician. Generally, an effective amount will be from about 0.01 to about 1,000, preferably from about 0.1 to about 250 and even more preferably from about 1 to about 50 mg per adult human per day.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three, four or more times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular composition thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of composition within the range that yields efficacy without

toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

The inhibitors and activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, or inhibitors and activators of β3 subunit transcription will be useful for treating a variety of diseases involving excessive or insufficient calcium sensitive potassium channel activity. Accordingly, the present invention includes a method of treating asthma, diabetes, glaucoma, pregnant human myometrium, cerebral ischemia, and conditions where stimulation of neurotransmitter release is desired such as Alzheimer's disease and stimulation of damaged nerves by administering to a patient a therapeutically effective amount of a substance that is an activator or an inhibitor of a calcium sensitive potassium channel containing a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein, or an activator or an inhibitor of β3 subunit transcription.

The modulators of channel function or transcription activity of the present invention are also expected to be useful in conditions where currently marketed inhibitors of potassium channels such as glyburide, glipizide, and tolbutamide are useful, *e.g.*, as antidiabetic agents. Calcium sensitive potassium channels contribute to the repolarization, and thus the de-excitation, of neurons. Thus, inhibitors of calcium sensitive potassium channels are expected to act as agents that tend to keep neurons in a depolarized, excited state. Many diseases, such as depression and memory disorders are thought to result from the impairment of neurotransmitter release. As agents that contribute to neuronal excitability, the inhibitors of the present invention are expected to useful in the treatment of such diseases since they will contribute to neuronal excitation and thus stimulate the release of neurotransmitters.

The activators of the present invention should be useful in conditions where it is desirable to decrease neuronal activity. Such conditions include, *e.g.*, excessive smooth muscle tone, angina, asthma, hypertension, incontinence, pre-term labor, migraine, cerebral ischemia, and Irritable Bowel Syndrome.

The calcium sensitive potassium channel subunits of the present invention are useful in conjunction with screens designed to identify activators and inhibitors of other ion channels. When screening compounds in order to identify

potential pharmaceuticals that specifically interact with a target ion channel, it is necessary to ensure that the compounds identified are as specific as possible for the target ion channel. To do this, it is necessary to screen the compounds against as wide an array as possible of ion channels that are similar to the target ion channel. Thus, in order to find compounds that are potential pharmaceuticals that interact with ion channel A, it is not enough to ensure that the compounds interact with ion channel A (the "plus target") and produce the desired pharmacological effect through ion channel A. It is also necessary to determine that the compounds do not interact with ion channels B, C, D, *etc.* (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). Human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, DNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, and recombinant cells that have been engineered to express human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins have utility in that they can be used as "minus targets" in screens designed to identify compounds that specifically interact with other ion channels. For example, Wang et al., 1998, Science 282:1890-1893 have shown that KCNQ2 and KCNQ3 form a heteromeric potassium ion channel know as the "M-channel." The M-channel is an important target for drug discovery since mutations in KCNQ2 and KCNQ3 are responsible for causing epilepsy (Biervert et al., 1998, Science 279:403-406; Singh et al., 1998, Nature Genet. 18:25-29; Schroeder et al., Nature 1998, 396:687-690). A screening program designed to identify activators or inhibitors of the M-channel would benefit greatly by the use of potassium channels comprising human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins as minus targets.

The present invention also includes antibodies to the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention can be raised against the entire human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or against suitable antigenic fragments of the subunit proteins that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and

Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or antigenic fragments, coupled to a suitable carrier, are injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice or chickens. The animals are bled periodically (or eggs collected) and sera obtained are tested for the presence of antibodies to the injected subunit or antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or antigenic fragments, coupled to a suitable carrier, are injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins into the cells of target organs. Nucleotides encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, lentivirus, and polio virus based vectors. Alternatively, nucleotides encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins will be particularly useful for the treatment of diseases where it is beneficial to elevate calcium sensitive potassium channel activity. cDNAs encoding mutant calcium

sensitive potassium channel subunits, that display a dominant negative phenotype, may be particularly useful for gene therapy treatment of diseases where it is beneficial to decrease calcium sensitive potassium channel activity.

The present invention includes processes for cloning orthologues of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits from non-human species. In general, such processes include preparing a PCR primer or a hybridization probe based upon SEQ.ID.NO.:1, 3, 5, 7, 9, or 20 that can be used to amplify a fragment containing the non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit (in the case of PCR) from a suitable DNA preparation or to select a cDNA or genomic clone containing the non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit from a suitable library. A preferred embodiment of this process is a process for cloning the calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit from mouse.

By providing DNA encoding mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, the present invention allows for the generation of an animal model of human diseases in which calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit activity is abnormal. Such animal models can be generated by making transgenic "knockout" or "knockin" mice containing altered calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes. Knockout mice can be generated in which portions of the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit gene have been deleted. Knockin mice can be generated in which mutations that have been shown to lead to human disease are introduced into the mouse gene. Such knockout and knockin mice will be valuable tools in the study of the relationship between calcium sensitive potassium channels and disease and will provide important model systems in which to test potential pharmaceuticals or treatments for human diseases involving calcium sensitive potassium channels.

Accordingly, the present invention includes a method of producing a transgenic mouse comprising:

(a) designing PCR primers or an oligonucleotide probe based upon SEQ.ID.NO.:1, 3, 5, 7, 9 or 20 for use in cloning the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit gene or cDNA;

(b) using the PCR primers or the oligonucleotide probe to clone at least a portion of the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c,

or β3d subunit gene or cDNA, the portion being large enough to use in making a transgenic mouse;

(c) producing a transgenic mouse having at least one copy of the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit gene altered from its native state.

Methods of producing knockout and knockin mice are well known in the art. One method involves the use of gene-targeted ES cells in the generation of gene-targeted transgenic knockout mice and is described in, *e.g.*, Thomas et al., 1987, Cell 51:503-512, and is reviewed elsewhere (Frohman et al., 1989, Cell 56:145-147; Capecchi, 1989, Trends in Genet. 5:70-76; Baribault et al., 1989, Mol. Biol. Med. 6:481-492).

Techniques are available to inactivate or alter any genetic region to virtually any mutation desired by using targeted homologous recombination to insert specific changes into chromosomal genes. Generally, use is made of a "targeting vector," *i.e.*, a plasmid containing part of the genetic region it is desired to mutate. By virtue of the homology between this part of the genetic region on the plasmid and the corresponding genetic region on the chromosome, homologous recombination can be used to insert the plasmid into the genetic region, thus disrupting the genetic region. Usually, the targeting vector contains a selectable marker gene as well.

In comparison with homologous extrachromosomal recombination, which occurs at frequencies approaching 100%, homologous plasmid-chromosome recombination was originally reported to only be detected at frequencies between 10^{-6} and 10^{-3} (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395; Smithies et al., 1985, Nature 317: 230-234; Thomas et al., 1986, Cell 44:419-428). Nonhomologous plasmid-chromosome interactions are more frequent, occurring at levels 10^5-fold (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395) to 10^2-fold (Thomas et al., 1986, Cell 44:419-428) greater than comparable homologous insertion.

To overcome this low proportion of targeted recombination in murine ES cells, various strategies have been developed to detect or select rare homologous recombinants. One approach for detecting homologous alteration events uses the polymerase chain reaction (PCR) to screen pools of transformant cells for homologous insertion, followed by screening individual clones (Kim et al., 1988, Nucleic Acids Res. 16:8887-8903; Kim et al., 1991, Gene 103:227-233). Alternatively, a positive genetic selection approach has been developed in which a

marker gene is constructed which will only be active if homologous insertion occurs, allowing these recombinants to be selected directly (Sedivy et al., 1989, Proc. Natl. Acad. Sci. USA 86:227-231). One of the most powerful approaches developed for selecting homologous recombinants is the positive-negative selection (PNS) method developed for genes for which no direct selection of the alteration exists (Mansour et al., 1988, Nature 336:348-352; Capecchi, 1989, Science 244:1288-1292; Capecchi, 1989, Trends in Genet. 5:70-76). The PNS method is more efficient for targeting genes which are not expressed at high levels because the marker gene has its own promoter. Nonhomologous recombinants are selected against by using the Herpes Simplex virus thymidine kinase (HSV-TK) gene and selecting against its nonhomologous insertion with herpes drugs such as gancyclovir (GANC) or FIAU (1-(2-deoxy 2-fluoro-B-D-arabinofluranosyl)-5-iodouracil). By this counter-selection, the percentage of homologous recombinants in the surviving transformants can be increased.

Other methods of producing transgenic mice involve microinjecting the male pronuclei of fertilized eggs. Such methods are well known in the art.

The present invention includes a transgenic, non-human animal in which the animal's genome contains DNA encoding at least a portion of a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit.

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Identification of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits and cDNA cloning

DNA sequence encoding the β1 subunit was used to search the GenBank database for homologous sequences encoding novel subunits. This search yielded an EST with similarity to β1 (AA904191). A cDNA encoding the EST was purchased (Genome Systems) and sequenced in both directions. Synthetic oligonucleotide primers (SEQ.ID.NOs.:12 and 13) were used to amplify the coding region and a small portion of the 3' untranslated region (UTR) of this gene (β2). The

coding region was then subcloned into a modified vector (pSP64T) containing an expanded polylinker between the 5' and 3' translation enhancer sequences (MVpl(+)).

The sequence of β2 was then used to search the GenBank database for additional novel beta subunits. The sequences from identified EST's were then used to search the database again. Several EST's were obtained in this iterative approach: AA195381, AA236930, AA236968, AA279911, AA761761, AA934876, AA195511, AA917510. The alignment of these sequences suggested they encoded the C-terminal portion of a novel β subunit, here designated β3. Available cDNAs encoding these ESTs were purchased (Genome Systems) and sequenced in both directions. None of these clones encoded full length protein based on the lack of 5' in-frame stop codons and amino acid alignments only to the middle of the first transmembrane segments of β1 and β2.

Unique and conserved portions of the individual subunits were used separately to search the databases for genomic sequences encoding these transcripts. A single 180 kilobase fragment of unidentified genomic sequence was identified using β3a, β3b and β3c specific fragments (GenBank accession number AC007823, version 2). Later versions of this entry contained a 40.4 kilobase contiguous fragment that contained all three specific fragments in the following order β3a, β3b and β3c. β3c is contiguous with the 5' end of the core sequence. See Figure 8.

A synthetic oligo, 5'-TTT ACA TTG TTA GTT TGC AGA CAG G-3' (SEQ.ID.NO.:19), annealing 3' of the β3 stop codon was used in a 5' RACE reaction as described in Clontech's Marathon Ready Spleen cDNA kit (catalog # 7412-1). This reaction yielded multiple products of varying sizes. Several fragments separated by electrophoresis were extracted from gel slices and cloned. Three distinct subunits were identified (β3a, β3b and β3c) in this manner.

To ensure novel subunits were not overlooked, the unfractionated product of the PCR amplification reaction was cloned directly into a TA cloning vector (pCR2.1, Invitrogen), without any attempt to isolate specific fragments. Colonies were then screened using a probe derived from EST AA761761 by the 'colony filter hybridization protocol' as described in *Current Protocols in Molecular Biology*, sections 6.1.1 and 6.3.1. DNA was prepared from hybridizing colonies. cDNAs with restriction digest patterns distinct from the original clones were sequenced in both directions. The open reading frames were determined and

amplified using synthetic oligonucleotide primers (SEQ.ID.NOs.:14 through 18), and subcloned into MVpl(+). One additional unique subunit was identified: β3d.

EXAMPLE 2

Analysis of expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits

Northern blot analysis: Northern blots containing poly(A+)-RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testes, ovary, small intestine, colon, and peripheral blood leukocytes were purchased from Clontech, Palo Alto, CA. The blots were probed with ^{32}P-labeled, randomly primed cDNA probes from β2 (nucleotides 268 to 1080 of SEQ.ID.NO.:1), β3a (nucleotides 70 to 384 of SEQ.ID.NO.:3), β3b (nucleotides 463 to 797 of SEQ.ID.NO.:5), and β3c/d (nucleotides 311 to 912 of SEQ.ID.NO.:7). The hybridization was carried out in 5X SSPE, 10X Denhardts solution, 50% Formamide, 2% SDS, 100ug/ml salmon sperm DNA at 42°C overnight. The washes were carried out stepwise in 2X SSC, 0.05% SDS at 42°C for 40 minutes, followed by 1X SSC, 0.05% SDS at 50°C for 40 minutes. High stringency washes were carried out at 0.1SSC, 0.05% SDS at 65°C for 40 minutes. Hybridization was detected either by exposure of the washed blots to X-ray film or by electronic detection using a phosphorimager.

Electrophysiological analysis: cRNAs were synthesized in vitro from plasmids encoding human *Slowpoke* α or the β2, β3a, β3b, β3c, or β3d subunits and injected into *Xenopus* oocytes (1.5 ng/oocyte of α subunit RNA +/- β subunit RNA at 1, 5, or 10X molar excess). Calcium sensitive potassium currents were recorded in inside-out patches. Recordings were performed under ionic conditions of symmetrical potassium. The standard pipette and bath solutions contained 116 mM potassium gluconate, 4 mM potassium chloride, 10 mM HEPES, pH 7.2. $CaCl_2$ was added to the bath solution to give final concentrations of free ionized calcium of 3-30 μM, taking into account the stability constant for calcium gluconate (15.9 M^{-1}). Currents were recorded using an EPC-7 amplifier (HEKA). The pClamp6.0 program (Axon Instruments) was used to generate voltage-clamp commands for data acquisition, and for analysis. NP_o - voltage relations were determined at 3, 10 and 30

μM bath calcium using two methods: (1) calculation of macroscopic conductance from peak or steady-state currents at test potentials (-80 to 80 mV), or (2) measurement or calculation of tail currents peaks (-80 mV) at test potentials. Boltzmann functions were fit to the data and used to derive the half-maximal activation parameter ($V_{1/2}$). Maximal inactivation parameters (30 μM Ca^{2+} and 80 mV) were calculated from current traces or averaged current traces. Inactivation rates were determined from single exponential fits. Fractional non-inactivating current was calculated as steady-state/peak current; fractional inactivating current was estimated as peak current minus steady-state current divided by peak current.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. An isolated DNA comprising nucleotides encoding a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein.

2. The DNA of claim 1 comprising nucleotides encoding a polypeptide having an amino acid sequence selected from the group consisting of: SEQ.ID.NO.:2; SEQ.ID.NO.:4; SEQ.ID.NO.:4 with an asparagine at position 163 instead of a serine; SEQ.ID.NO.:6; SEQ.ID.NO.:6 with a serine at position 143 instead of an asparagine; SEQ.ID.NO.:8; SEQ.ID.NO.:8 with an asparagine at position 161 instead of a serine; SEQ.ID.NO.:10; SEQ.ID.NO.:10 with a serine at position 165 instead of an asparagine;and positions 2-246 of SEQ.ID.NO.:6.

3. The DNA of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ.ID.NO.:1, 3, 5, 7, 9, and 20.

4. The DNA of claim 1 comprising a nucleotide sequence selected from the group consisting of: positions 271-975 of SEQ.ID.NO.:1, positions 341 to 1171 of SEQ.ID.NO.:3, positions 796 to 1566 of SEQ.ID.NO.:5, positions 869 to 1693 of SEQ.ID.NO.:7, and positions 457 to 1293 of SEQ.ID.NO.:9.

5. An isolated DNA that hybridizes under stringent conditions to the DNA of claim 2.

6. An expression vector comprising the DNA of claim 1.

7. A recombinant host cell comprising the DNA of claim 1.

8. An isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein.

9. The protein of claim 8 having an amino acid sequence selected from the group consisting of: SEQ.ID.NO.:2; SEQ.ID.NO.:4; SEQ.ID.NO.:4 with an asparagine at position 163 instead of a serine; SEQ.ID.NO.:6; SEQ.ID.NO.:6 with a

serine at position 143 instead of an asparagine; SEQ.ID.NO.:8; SEQ.ID.NO.:8 with an asparagine at position 161 instead of a serine; SEQ.ID.NO.:10; SEQ.ID.NO.:10 with a serine at position 165 instead of an asparagine; and positions 2-246 of SEQ.ID.NO.:6.

10. The protein of claim 8 containing a single amino acid substitution.

11. The protein of claim 8 containing two or more amino acid substitutions where the amino acid substitutions do not occur in conserved positions.

12. A polypeptide having at least 80% sequence identity to the protein of claim 9 when measured by BLAST or FASTA.

13. An antibody that binds specifically to a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein; or that binds specifically to the β3 subunit family of proteins by binding to the conserved core.

14. A DNA or RNA oligonucleotide probe comprising at least 15 contiguous nucleotides of at least one of a sequence selected from the group consisting of: SEQ.ID.NO.:1, 3, 5, 7, 9, and 20.

15. A method for identifying substances that bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing cells expressing a calcium sensitive potassium channel containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(b) exposing the cells to a substance that is not known to bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) determining the amount of binding of the substance to the cells;

(d) comparing the amount of binding in step (c) to the amount of binding of the substance to control cells where the control cells are substantially

identical to the cells of step (a) except that the control cells do not express human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the amount of binding in step (c) is greater than the amount of binding of the substance to control cells, then the substance binds to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

16. A method of identifying substances that bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and thus are likely to be inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing cells expressing calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(b) exposing the cells to a compound that is known to bind to the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) determining the amount of binding of the compound to the cells in the presence and in the absence of a substance not known to bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the amount of binding of the compound in the presence of the substance differs from that in the absence of the substance, then the substance binds calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and is likely to be an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

17. A method of identifying activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) recombinantly expressing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or mutant human calcium

sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a host cell so that the recombinantly expressed human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins form calcium sensitive potassium channels by forming heteromers with other calcium sensitive potassium channel subunit proteins;

(b) measuring the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence and in the absence of a substance suspected of being an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where a change in the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

18. A method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector;

(c) comparing the abundance of the reporter protein in the cells of step (b) to the abundance of the reporter protein in cells transfected with the vector without fragments of the promoter region of the β3 gene;

where fragments of the promoter region of the β3 gene which increase the abundance of the reporter protein in the absence of other promoter elements only in cells which endogenously express β3a, β3b, β3c, or β3d subunits are promoter elements; sequences which decrease the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which do not endogenously express β3a, β3b, β3c, or β3d subunits are repressor elements; and sequences which increase the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which endogenously express β3a, β3b, β3c, or β3d subunits are enhancer elements.

19. The method of claim 18 where the vector contains promoter or enhancer sequence elements which function independently of the fragments of the promoter region of the β3 gene.

20. The method of claim 18 where the abundance of the reporter protein is normalized with respect to the fraction of transfected cells.

21. A method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells; and

(b) separating the incubation on a gel;

where fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene that migrate differently in a gel ('undergo a shift') after incubation with nuclear extracts from cells are DNA sequences which bind nuclear factors which promote, enhance or repress β3 gene expression.

22. The method of claim 21 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the method of claim 18.

23. The method of claim 21 where the cells express β3a, β3b, β3c, or β3d subunits.

24. The method of claim 21 where the cells do not express β3a, β3b, β3c, or β3d subunits.

25. A method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene

(SEQ.ID.NO.:20, nucleotides 1 to 17,436) with cloned or purified transcription factors and separating the incubation on a gel;

where factors which bind β3 gene promoter sequence elements will induce a shift in the migration of the radiolabeled DNA fragments, and are involved in β3 gene transcription regulation.

26. The method of claim 25 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

27. A method of identifying transcription factors involved in β3 gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells and separating the incubation on a gel;

(b) adding an antibody that specifically recognizes a single transcription factor or a family of transcription factors to the incubation of step (a), followed by separating the incubation on a gel;

where a super-shift in mobility of the double stranded DNA in step (b) as compared to step (a) indicates that a transcription factor recognized by the antibody binds the double stranded DNA.

28. A method of identifying clones encoding nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) screening an expression library with radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436)

(b) determining which clones of the library bind the radiolabeled fragments of double stranded DNA;

(c) amplifying and sequencing the clones of step (b).

29. The method of claim 28 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

30. A method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating phage expressing cDNA encoded fusion proteins at their surface with the matrix;

(c) removing phage that do not bind to the matrix by washing;

(d) eluting phage bound to the matrix with excess fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

where the phage eluted in step (d) encode nuclear factors involved in β3 gene transcription regulation.

31. The method of claim 30 where the DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

32. The method of claim 30 where the phage eluted at step (d) are amplified and sequenced.

33. A method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating nuclear extracts from cells with the matrix;

(c) washing non-binding proteins from the nuclear extract from the matrix;

(d) eluting bound proteins from the matrix with excess double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

where the eluted proteins from step (d) are nuclear factors involved in β3 gene transcription regulation.

34. The method of claim 33 further comprising separating the eluted proteins from step (d) on a gel and staining the gel to test for purity of the eluted proteins.

35. The method of claim 34 further comprising sequencing the proteins that have been separated on the gel.

36. The method of claim 34 further comprising immunological analysis of the proteins that have been separated on the gel with antibodies directed towards known transcription factors to identify the eluted proteins by western blot or immunoprecipitation.

37. The method of claim 33 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

38. A method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) constructing a yeast strain that contains a few to several copies of a fragment of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) preceding a cDNA encoding a reporter protein;

(b) constructing a cDNA library from cells in a vector that allows formation of fusion proteins encoded by the inserted cDNA and a transcription activation domain;

(c) transforming the library of (b) into the yeast strain of (a) and isolating colonies of yeast displaying expression of the reporter protein.

39. The method of claim 38 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

40. The method of claim 38 further comprising purifying the vectors from the isolated colonies and sequencing the cDNA in the vectors.

41. A method of identifying substances that enhance or inhibit the rate of transcription of the β3 gene comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ.ID.NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector in the presence and absence of a compound;

where (1) if the presence of the compound decreases the abundance of the reporter protein, then the compound is a substance that inhibits the rate of transcription of the β3 gene; (2) if the presence of the compound increases the abundance of the reporter protein, then the compound is a substance that enhances the rate of transcription of the β3 gene.

42. The method of claim 41 further comprising a control in which the effect of the compound on the abundance of the reporter protein in control cells is measured, where the control cells are cells that are essentially the same as the cells of step (b) except that the control cells have been transfected with a vector that lacks fragments of the promoter region of the β3 gene.

FIGURE 1A

```
   1 CTTAATCCTA TCCAAGTATG CAGTACGCTC TTGGGTCGTC TCATGAGACC CAGGGGCATG
  61 TTGGAAAGAA CTGAGAGAAA GAGCAACAAA GCGGCGAGTG GTGTGAGAGG GCAGCACGCG
 121 CTGTGGGGCC CTTCCAGAGA AATGTACTGA AAAAGTCTAC GCAATGTCTG GGATTTGCTA
 181 AACAATACCT GGAAAGCAGA CAGGTTTTTT TGCCATTCCT CCAGGACATC CACCATAAGG
 241 AAAGGAGACC CTGGACCAAC ATTCTCTAAG ATGTTTATAT GGACCAGTGG CCGGACCTCT
 301 TCATCTTATA GACATGATGA AAAAAGAAAT ATTTACCAGA AAATCAGGGA CCATGACCTC
 361 CTGGACAAAA GGAAAACAGT CACAGCACTG AAGGCAGGAG AGGACCGAGC TATTCTCCTG
 421 GGACTGGCTA TGATGGTGTG CTCCATCATG ATGTATTTTC TGCTGGGAAT CACACTCCTG
 481 CGCTCATACA TGCAGAGCGT GTGGACCGAA GAGTCTCAAT GCACCTTGCT GAATGCGTCC
 541 ATCACGGAAA CATTTAACTG CTCCTTCAGC TGTGGTCCAG ACTGCTGGAA ACTTTCTCAG
 601 TACCCCTGCC TCCAGGTGTA CGTTAACCTG ACTTCTTCCG GGGAAAAGCT CCTCCTCTAC
 661 CACACAGAAG AGACAATAAA AATCAATCAG AAGTGCTCCT ATATACCTAA ATGTGGAAAA
 721 AATTTTGAAG AATCCATGTC CCTGGTGAAT GTTGTCATGG AAAACTTCAG GAAGTATCAA
 781 CACTTCTCCT GCTATTCTGA CCCAGAAGGA AACCAGAAGA GTGTTATCCT AACCAAACTC
 841 TACAGTTCCA ACGTGCTGTT CCATTCACTC TTCTGGCCAA CCTGTATGAT GGCTGGGGGT
 901 GTGGCAATTG TTGCCATGGT GAAACTTACA CAGTACCTCT CCCTACTATG TGAGAGGATC
 961 CAACGGATCA ATAGATAAAT GCAAAAATGG ATAAAATAAT TTTTGTTAAA GCTCAAATAC
1021 TGTTTTCTTT CATTCTTCAC CAAAGAACCT TAAGTTTGTA ACGTGCAGTC TGTTATGAGT
1081 TCCCTAATAT ATTCTTATAT GTAGAGCAAT AATGCAAAAG CTGTTCTATA TGCAAACATG
1141 ATGTCTTTAT TATTCAGGAG AATAAATAAC TGTTTTGTGT TGAA
```

FIGURE 1B

```
  1 MFIWTSGRTS SSYRHDEKRN IYQKIRDHDL LDKRKTVTAL KAGEDRAILL GLAMMVCSIM
 61 MYFLLGITLL RSYMQSVWTE ESQCTLLNAS ITETFNCSFS CGPDCWKLSQ YPCLQVYVNL
121 TSSGEKLLLY HTEETIKINQ KCSYIPKCGK NFEESMSLVN VVMENFRKYQ HFSCYSDPEG
181 NQKSVILTKL YSSNVLFHSL FWPTCMMAGG VAIVAMVKLT QYLSLLCERI QRINR
```

FIGURE 2A

```
   1 GCTCCCGGCT GCCGAGGCGG AAACACAGGT GATGAGGTGG CGGCAAGCAC AGTGCAAAGA
  61 GAGAGAAGCA GCTTCGGCTG CAGCAAACCA CGCAGGTCCT TCTTGATCAT CTAGAACTGA
 121 CCGCTCCGCC TTGCCAGGAG TCTGCAGAAC CACGTGGCTG GCCTGCCTGA AGTTCTCACC
 181 TCTCTAGGAA GGCGGGGGGC TTCTAATGGC TGCAGCTGCG CTGGGGGCTG GGGGCTCCCG
 241 CTGGGACTCC ACTTCCGTGG ATGTCTAAGC TTCACCTTTC TTGCGCCCGC AGGGGCATGA
 301 CTCAGGTGAA AGGGAGCCAT TTTCTCAGAC CCCTGGCCTC ATGCAGCCCT TCAGCATCCC
 361 CGTGCAAATC ACACTTCAGG GCAGCCGGAG GCGCCAGGGG AGGACAGCCT TTCCTGCCTC
 421 AGGGAAGAAG AGAGAGACAG ACTACAGTGA TGGAGACCCA CTAGATGTGC ACAAGAGGCT
 481 GCCATCCAGT ACTGGAGAGG ACCGAGCCGT GATGCTGGGG TTTGCCATGA TGGGCTTCTC
 541 AGTCCTAATG TTCTTCTTGC TCGGAACAAC CATTCTAAAG CCTTTTATGC TCAGCATTCA
 601 GAGAGAAGAA TCGACCTGCA CTGCCATCCA CACAGATATC ATGGACGACT GGCTGGACTG
 661 TGCCTTCACC TGTGGTGTGC ACTGCCACGG TCAGGGGAAG TACCCGTGTC TTCAGGTGTT
 721 TGTGAACCTC AGCCATCCAG GTCAGAAAGC TCTCCTACAT TATAATGAAG AGGCTGTCCA
 781 GATAAATCCC AAGTGCTTTT ACACACCTAA GTGCCACCAA GATAGAAGTG ATTTGCTCAA
 841 CAGTGCTCTG GACATAAAAG AATTCTTCGA TCACAAAAAT GGAACCCCCT TTTCATGCTT
 901 CTACAGTCCA GCCAGCCAAT CTGAAGATGT CATTCTTATA AAAAAGTATG ACCAAATGGC
 961 TATCTTCCAC TGTTTATTTT GGCCTTCACT GACTCTGCTA GGTGGTGCCC TGATTGTTGG
1021 CATGGTGAGA TTAACACAAC ACCTGTCCTT ACTGTGTGAA AAATATAGCA CTGTAGTCAG
1081 AGATGAGGTA GGTGGAAAAG TACCTTATAT AGAACAGCAT CAGTTCAAAC TGTGCATTAT
1141 GAGGAGGAGC AAAGGAAGAG CAGAGAAATC TTAAGACGGT GGCCAAATTA AAGTGCTGGC
1201 CTTCAGATGT CTGTGATTTC TGCAACTCGA GTATGCG
```

FIGURE 2B

```
  1 MQPFSIPVQI TLQGSRRRQG RTAFPASGKK RETDYSDGDP LDVHKRLPSS TGEDRAVMLG
 61 FAMMGFSVLM FFLLGTTILK PFMLSIQREE STCTAIHTDI MDDWLDCAFT CGVHCHGQGK
121 YPCLQVFVNL SHPGQKALLH YNEEAVQINP KCFYTPKCHQ DRSDLLNSAL DIKEFFDHKN
181 GTPFSCFYSP ASQSEDVILI KKYDQMAIFH CLFWPSLTLL GGALIVGMVR LTQHLSLLCE
241 KYSTVVRDEV GGKVPYIEQH QFKLCIMRRS KGRAEKS
```

FIGURE 3A

```
   1 AAGAGAAAGA ACAAGAAAAA GAAAAAGAAG AGGAAAAAAT CCCCAGTACC CATAGAAACC
  61 CTTAAAGATG TTTAAAAAGA GTTAACTTAT CAGAACACAG ATTTAAGTGA AATTAAGGAA
 121 GAAGAGCAGG TAAAGTCTAC TGACAGAAAG TCAGCAGTGG AAGCCCAAAA CGAGGTGACT
 181 GAAAATCCAA AACAGAAAAT TGCAGCAGAA AGCAGTGAAA ATGCTGATTG TCCAGAGAAT
 241 CCTAAAATGA AGTTGGATGG AAAACTTGAC CAAGAAGGCA ATGATGTAAA AACAGCAGCT
 301 GAGGAGGTAC TAGCTGGTAG AGACACATTA GATTTTGAGG ATGTCACAGT TCAATCATCA
 361 GGCCCGAGGG CTGGTGGTGA AGAATTAGAT GAAGGTGTTG CAAAAGATAA TGCTAAAATA
 421 GCTGGTGCCA CTTAAAGCAA TCCTGAAGAA CCAGAGAGTG AAGATGCAGA TCACTGCACC
 481 GTACCCAAAA ATGAAAGTCC CTCACAGGAC ATTAGTGATG CCTGTGAAGC AGAAAGTACA
 541 GAGAGGTGCG GGATGTCAGA ACATCCAAGT CAGACCATCA GGAAAGCTTT AGACAGCAAT
 601 AGCCTAAAAA ACCATGACTT GTTGGCACCA GGAGGAGAGC CGGGGGACTT CAATCCAGAA
 661 AGCAGAGAAG ATACCAGAGG AGGGAACGAG AAGGGCAAAA GCAAAGAAGA CCGTACCATG
 721 TCCTAAGCTG AGGCAGGCGG CAGGCGTGGT GCACAGGAAG TCTGAGTGTG AGGGGCTCTT
 781 TTCTCTCCAC TGCCAATGAC AGCCTTTCCT GCCTCAGGGA AGAAGAGAGA GACAGACTAC
 841 AGTGATGGAG ACCCACTAGA TGTGCACAAG AGGCTGCCAT CCAGTACTGG AGAGGACCGA
 901 GCCGTGATGC TGGGGTTTGC CATGATGGGC TTCTCAGTCC TAATGTTCTT CTTGCTCGGA
 961 ACAACCATTC TAAAGCCTTT TATGCTCAGC ATTCAGAGAG AAGAATCGAC CTGCACTGCC
1021 ATCCACACAG ATATCATGGA CGACTGGCTG GACTGTGCCT TCACCTGTGG TGTGCACTGC
1081 CACGGTCAGG GGAAGTACCC GTGTCTTCAG GTGTTTGTGA ACCTCAGCCA TCCAGGTCAG
1141 AAAGCTCTCC TACATTATAA TGAAGAGGCT GTCCAGATAA ATCCCAAGTG CTTTTACACA
1201 CCTAAGTGCC ACCAAGATAG AAATGATTTG CTCAACAGTG CTCTGGACAT AAAAGAATTC
1261 TTCGATCACA AAAATGGAAC CCCCTTTTCA TGCTTCTACA GTCCAGCCAG CCAATCTGAA
1321 GATGTCATTC TTATAAAAAA GTATGACCAA ATGGCTATCT TCCACTGTTT ATTTTGGCCT
1381 TCACTGACTC TGCTAGGTGG TGCCCTGATT GTTGGCATGG TGAGATTAAC ACAACACCTG
1441 TCCTTACTGT GTGAAAAATA TAGCACTGTA GTCAGAGATG AGGTAGGTGG AAAAGTACCT
1501 TATATAGAAC AGCATCAGTT CAAACTGTGC ATTATGAGGA GGAGCAAAGG AAGAGCAGAG
1561 AAATCTTAAG ACGGTGGCCA AATTAAAGTG CTGGCCTTCA GATGTCTGTG ATTTCTGCAA
1621 CTCGAGTATG CG
```

FIGURE 3B

```
  1 MTAFPASGKK RETDYSDGDP LDVHKRLPSS TGEDRAVMLG FAMMGFSVLM FFLLGTTILK
 61 PFMLSIQREE STCTAIHTDI MDDWLDCAFT CGVHCHGQGK YPCLQVFVNL SHPGQKALLH
121 YNEEAVQINP KCFYTPKCHQ DRNDLLNSAL DIKEFFDHKN GTPFSCFYSP ASQSEDVILI
181 KKYDQMAIFH CLFWPSLTLL GGALIVGMVR LTQHLSLLCE KYSTVVRDEV GGKVPYIEQH
241 QFKLCIMRRS KGRAEKS
```

FIGURE 4A

```
   1 CCCAGCTACT CGGGAGGCTG AGGCAGGAGA ATCGCTTGAA CCTGGGAGGC GGAGGAGGTT
  61 GCAGTGAACT GAGATCGTAC CCAGCCTGGG CAACAGTGCG AGGCTCCGTC TCAAAAAAAA
 121 ACCAAAAAAC ACAAAAACAA AAAACGACAG AGAAGGCCAA ACAAAACACA TCTGTGGGCT
 181 GGATGCCGCC ATGCCCACCG GTTTGCGACC TTTGTGTTGG ACTCTTCTGT TCACCAGACA
 241 CCCTGCCCTG CGAGAATGTA TCTCATCCTT TGCTGGAGCA GGTTTGCAGG CACAGTGGAG
 301 AGAGGAGAGA AGAAATGAAG GGACACTTAT GCAGAACCAT GAGTGGCCAG AGAGGAGGAG
 361 AAGGAGGGTG AGAGGAGCAA AGAAGCCATG ACAACTTCAT AATTCTGAGT GGACTGGGCA
 421 GTGGCCAGAA ATTCTGGTGG TGGATATGCT GCCTTTCCAA CAGGTGAATA TGAAAGAATA
 481 AGTCAAACCC TGTTCAGGAC GCTGTTAATT CCAAATGTGA ACTTTTTGAG TCATTCTTTT
 541 CATGTGGAAT TCAAAGGAGA ATGTAAACAA ATTTTCAGGA GGGACGTGCA ATATCCCTGA
 601 AAGATAACAG AGTTCGTAAC ACTTATTTAC ATACAACATT CTCTAGTTAT TGATTAAACA
 661 GATCTCTACA GACTTGCATG AGGCAACATT TCTTAGGCTT GTTTGCTACA ATATCTTTAA
 721 AAATACTTGA TTACACATCA CTTTAGCTTA TTTAGATGGA CTTTTCACCA AGCTCTGAAC
 781 TGGGATTTCA TTTTGTTGCA TTCATCCTGC TCACGAGACA CAGGTAGGCA GCAAATGAGA
 841 TTATCCCTCC AGTCCCCATG GATTGGAAAT GTTCCCCCTT CTTTATGAGC TCACTGCAGT
 901 ATCTCCTTCT CCCTTTCCCC AAAGGACAGC CTTTCCTGCC TCAGGGAAGA AGAGAGAGAC
 961 AGACTACAGT GATGGAGACC CACTAGATGT GCACAAGAGG CTGCCATCCA GTACTGGAGA
1021 GGACCGAGCC GTGATGCTGG GGTTTGCCAT GATGGGCTTC TCAGTCCTAA TGTTCTTCTT
1081 GCTCGGAACA ACCATTCTAA AGCCTTTTAT GCTCAGCATT CAGAGAGAAG AATCGACCTG
1141 CACTGCCATC CACACAGATA TCATGGACGA CTGGCTGGAC TGTGCCTTCA CCTGTGGTGT
1201 GCACTACCAC GGTCAGGGGA AGTACCCGTG TCTTCAGGTG TTTGTGAACC TCAGCCATCC
1261 AGGTCAGAAA GCTCTCCTAC ATTATAATGA AGAGGCTGTC CAGATAAATC CCAAGTGCTT
1321 TTACACACCT AAGTGCCACC AAGATAGAAG TGATTTGCTC AACAGTGCTC TGGACATAAA
1381 AGAATTCTTC GATCACAAAA ATGGAACCCC CTTTTCATGC TTCTACAGTC CAGCCAGCCA
1441 ATCTGAAGAT GTCATTCTTA TAAAAAAGTA TGACCAAATG GCTATCTTCC ACTGTTTATT
1501 TTGGCCTTCA CTGACTCTGC TAGGTGGTGC CCTGATTGTT GGCATGGTGA GATTAACACA
1561 ACACCTGTCC TTACTGTGTG AAAAATATAG CACTGTAGTC AGAGATGAGG TAGGTGGAAA
1621 AGTACCTTAT ATAGAACAGC ATCAGTTCAA ACTGTGCATT ATGAGGAGGA GCAAAGGAAG
1681 AGCAGAGAAA TCTTAAGACG GTGGCCAAAT TAAAGTGCTG GCCTTCAGAT GTCTGTGATT
1741 TCTGCAACTC GAGTATGCG
```

FIGURE 4B

```
  1 MFPLLYELTA VSPSPFPQRT AFPASGKKRE TDYSDGDPLD VHKRLPSSTG EDRAVMLGFA
 61 MMGFSVLMFF LLGTTILKPF MLSIQREEST CTAIHTDIMD DWLDCAFTCG VHCHGQGKYP
121 CLQVFVNLSH PGQKALLHYN EEAVQINPKC FYTPKCHQDR SDLLNSALDI KEFFDHKNGT
181 PFSCFYSPAS QSEDVILIKK YDQMAIFHCL FWPSLTLLGG ALIVGMVRLT QHLSLLCEKY
241 STVVRDEVGG KVPYIEQHQF KLCIMRRSKG RAEKS
```

FIGURE 5A

```
   1 CGCCGCGGAT CCGAAATGAA GGGACACTTA TGCAGAACCA TGAGTGGCCA GAGAGGAGGA
  61 GAAGGAGGGT GAGAGGAGCA AAGAAGCCAT GACAACTTCA TAATTCTGAG TGGACTGGGC
 121 AGTGGCCAGA AATTCTGGTG GTGGATATGC TGCCTTTCCA ACAGGTGAAT ATGAAAGAAT
 181 AAGTCAAACC CTGTTCAGGA CGCTGTTAAT TCCAAATGTG AACTTTTTGA GTCATTCTTT
 241 TCATGTGGAA TTCAAAGGAG AATGTAAACA AATTTTCAGG AGGGACGTGC AATATCCCTG
 301 AAAGATAACA AAGTTCGTAA CACTTATTTA CATACAACAT TCTCTAGTTA TTGATTAAAC
 361 AGATCTCTAC AGACTTGCAT GAGGCAACAT TTCTTAGGCT TGTTTGCTAC AATATCTTTA
 421 AAAATACTTG ATTACACATC ACTTTAGCTT ATTTAGATGG ACTTTTCACC AAGCTCTGAA
 481 CTGGGATTTC ATTTTGTTGC ATTCATCCTG CTCACGAGAC ACAGGACAGC CTTTCCTGCC
 541 TCAGGGAAGA AGAGAGAGAC AGACTACAGT GATGGAGACC CACTAGATGT GCACAAGAGG
 601 CTGCCATCCA GTACTGGAGA GGACCGAGCC GTGATGCTGG GGTTTGCCAT GATGGGCTTC
 661 TCAGTCCTAA TGTTCTTCTT GCTCGGAACA ACCATTCTAA AGCCTTTTAT GCTCAGCATT
 721 CAGAGAGAAG AATCGACCTG CACTGCCATC CACACAGATA TCATGGACGA CTGGCTGGAC
 781 TGTGCCTTCA CCTGTGGTGT GCACTGCCAC GGTCAGGGGA AGTACCCGTG TCTTCAGGTG
 841 TTTGTGAACC TCAGCCATCC AGGTCAGAAA GCTCTCCTAC ATTATAATGA AGAGGCTGTC
 901 CAGATAAATC CCAAGTGCTT TTACACACCT AAGTGCCACC AAGATAGAAA TGATTTGCTC
 961 AACAGTGCTC TGGACATAAA AGAATTCTTC GATCACAAAA ATGGAACCCC CTTTTCATGC
1021 TTCTACAGTC CAGCCAGCCA ATCTGAAGAT GTCATTCTTA TAAAAAAGTA TGACCAAATG
1081 GCTATCTTCC ACTGTTTATT TTGGCCTTCA CTGACTCTGC TAGGTGGTGC CCTGATTGTT
1141 GGCATGGTGA GATTAACACA ACACCTGTCC TTACTGTGTG AAAAATATAG CACTGTAGTC
1201 AGAGATGAGG TAGGTGGAAA AGTACCTTAT ATAGAACAGC ATCAGTTCAA ACTGTGCATT
1261 ATGAGGAGGA GCAAAGGAAG AGCAGAGAAA TCTTAA
```

FIGURE 5B

```
  1 MDFSPSSELG FHFVAFILLT RHRTAFPASG KKRETDYSDG DPLDVHKRLP SSTGEDRAVM
 61 LGFAMMGFSV LMFFLLGTTI LKPFMLSIQR EESTCTAIHT DIMDDWLDCA FTCGVHCHGQ
121 GKYPCLQVFV NLSHPGQKAL LHYNEEAVQI NPKCFYTPKC HQDRNDLLNS ALDIKEFFDH
181 KNGTPFSCFY SPASQSEDVI LIKKYDQMAI FHCLFWPSLT LLGGALIVGM VRLTQHLSLL
241 CEKYSTVVRD EVGGKVPYIE QHQFKLCIMR RSKGRAEKS
```

FIGURE 6

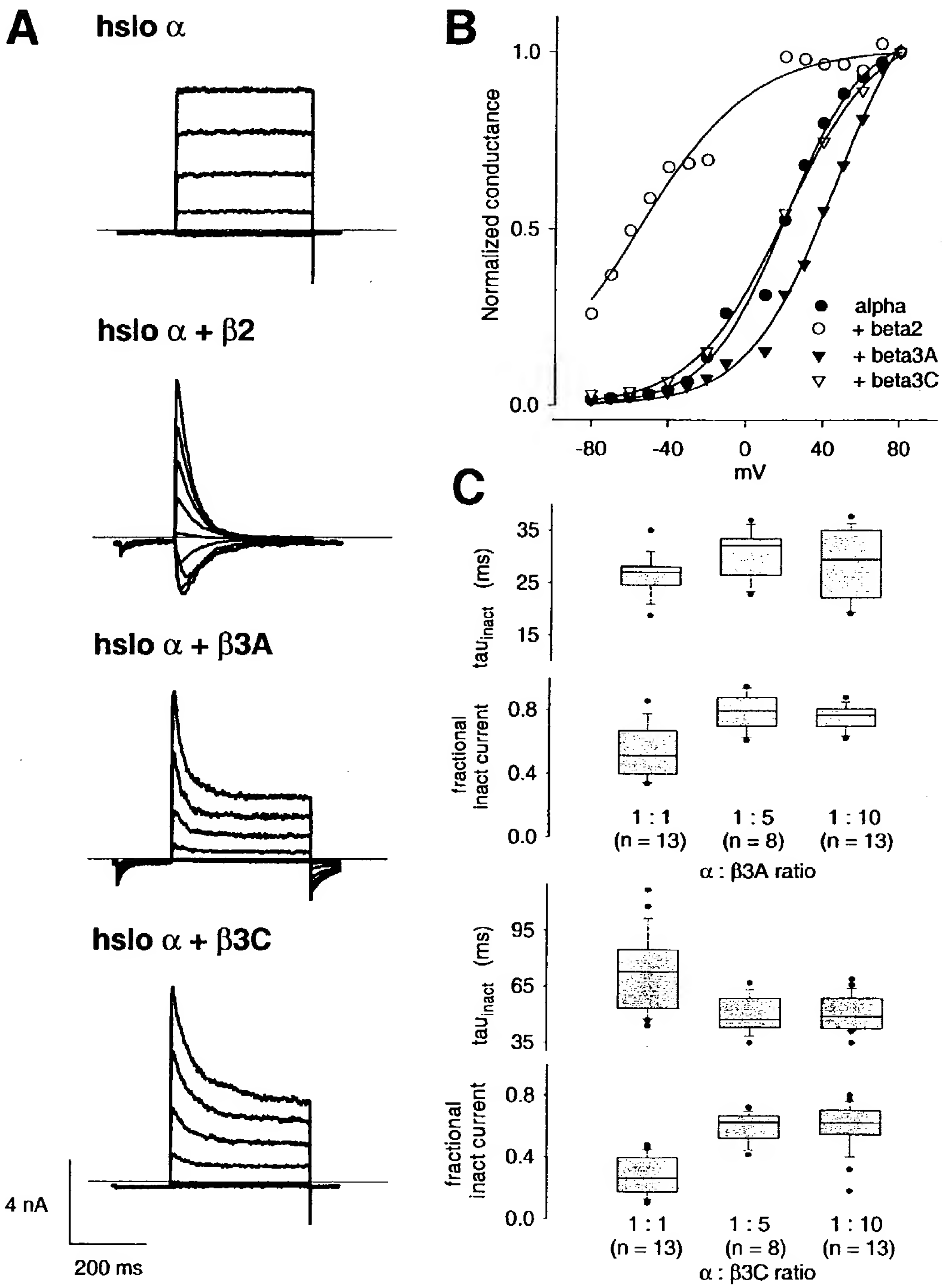
```
BKb1   ....................................................MVK.KLVM
BKb2   ......................MFIWTSGRTSSSYRHDEKRNIYQKIRDHDLLDKRKTVT
BKb3a  .........MQPFSIPVQITLQGSRRRQGRTAFPASGKKRETDYS...DGDPLDVHKRLP
BKb3b  .............................MTAFPASGKKRETDYS...DGDPLDVHKRLP
BKb3c  ...........MFPLLYELTAVSPSPFPQRTAFPASGKKRETDYS...DGDPLDVHKRLP
BKb3d  MDFSPSSELGFHFVAFILLTRH.......RTAFPASGKKRETDYS...DGDPLDVHKRLP

BKb1   AQKRGETRALCLGVTMVVCAVITYYILVTTVLPLYQKSVWTQESKCHLI......ET.NI
BKb2   ALKAGEDRAILLGLAMMVCSIMMYFLLGITLLRSYMQSVWTEESQCTLLNASIT.ETFNC
BKb3a  SS.TGEDRAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC
BKb3b  SS.TGEDRAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC
BKb3c  SS.TGEDRAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC
BKb3d  SS.TGEDRAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC

BKb1   RDQEELKGKKVPQYPCL..WVNVS.AAGRWAVLYHTEDTRDQNQQCSYIPGSV..DNYQT
BKb2   SFSCGPDCWKLSQYPCLQVYVNLTSS.GEKLLLYHTEETIKINQKCSYIPKCG..KNFEE
BKb3a  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRNDLL
BKb3b  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRNDLL
BKb3c  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRSDLL
BKb3d  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRSDLL

BKb1   ARADVEKVRAKFQEQQVFYCFSAPRGNETSVLFQRLYGPQALLFSLFWPTFLLTGGLLII
BKb2   SMSLVNVVMENFRKYQHFSCYSDPEGNQKSVILTKLYSSNVLFHSLFWPTCMMAGGVAIV
BKb3a  NSALDIKEFFDHKNGTPFSCFYSPASQSEDVILIKKYDQMAIFHCLFWPSLTLLGGALIV
BKb3b  NSALDIKEFFDHKNGTPFSCFYSPASQSEDVILIKKYDQMAIFHCLFWPSLTLLGGALIV
BKb3c  NSALDIKEFFDHKNGTPFSCFYSPASQSEDVILIKKYDQMAIFHCLFWPSLTLLGGALIV
BKb3d  NSALDIKEFFDHKNGTPFSCFYSPASQSEDVILIKKYDQMAIFHCLFWPSLTLLGGALIV

BKb1   AMVKSNQYLSILAAQK...................................
BKb2   AMVKLTQYLSLLCERIQRINR..............................
BKb3a  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGRAEKS
BKb3b  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGRAEKS
BKb3c  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGRAEKS
BKb3d  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGRAEKS
```



A
hslo α
hslo α + β2
hslo α + β3A
hslo α + β3C
4 nA
200 ms
B
Normalized conductance
1.0
0.5
0.0
-80
-40
0
40
80
mV
alpha
+ beta2
+ beta3A
+ beta3C
C
tau_{inact} (ms)
35
25
15
fractional inact current
0.8
0.4
0.0
1 : 1 (n = 13)
1 : 5 (n = 8)
1 : 10 (n = 13)
α : β3A ratio
tau_{inact} (ms)
95
65
35
fractional inact current
0.8
0.4
0.0
1 : 1 (n = 13)
1 : 5 (n = 8)
1 : 10 (n = 13)
α : β3C ratio

Figure 7

FIGURE 8A

```
   1 aatcatgtga gttcatcatt ataaaacagc tgatttataa caactgattt taaagtgggt
  61 tgttagcttg aagataaatg agatagggca tgattcaggt ttctgtgtac acactatatt
 121 ctaatgaaag taattacaat tatcttctaa acagctgcag atattttttc tataaacaat
 181 tttagatgtt acattgttaa atgttagctt tcaaattctt cacattttaa ctcaatgaag
 241 tcctttttag caaacttaca ggaatcattg tatcattcag ctattaaata aggaattgtt
 301 ctaattcaca tcttaattaa gaactttact aggttatatc ttttgcaagt atgagaataa
 361 tctagataga aggactagaa ttggattaaa ggtgatccag ataaaaatag taattctaat
 421 gaggaaattt tttaacattg aaaatagtac cctgtttatt ttttttaatg ttagagtcaa
 481 accattgcac attactgtgt aaaaatacac aaagatggta acttgctgct aaagcccttt
 541 ttacttttga atctttgtat ttttttcacc tttgtaattt taagttgtgc ttttatcact
 601 catttgtttt ctatctttat ttctgtttgg gttaaagtta caaacagcaa gtttttgtgt
 661 ttaaattcct gaaaatgttg cgatggaatt acagtaatgt gttacggctt gggcccctcg
 721 caggagtgta tctcgcagtc agcagtgaaa acaaagttcg aacagcacac tatcagagct
 781 aaacagatac tagctactgt gaaaaacata atggattcag taaacctggc agctgaagat
 841 aaaaggtatg agttcatttt gttgcaacat aaaaatgtta gttttttttgt cagacttttg
 901 ttaagatgag gttttaaatt gttgtactat aaatactttt tacactgaaa tcaaccttgt
 961 gggtttaact gatgcctact tctatatttt taagtgcgta tttgaaagtg tcgatattta
1021 ttctgcaacc ttgcacacat catttatatc ccctggacct cactattcct gtgtatgaat
1081 tgtgattttt aaaatgtttt gtggctttaa aatatctttg aagcagtgga agaaccattt
1141 cttcaagcag atttttatga agaagtccaa aacaggtgat cctctttctg cattcaaccc
1201 acgacccatt ttttaatatg gtcatacatt ggtattttga aatcactatt ttatactagt
1261 tgtgtttgtt tattgcctgg tgaacaaaca aggcgatgct acctgccaac cagaaagttg
1321 atttggtaag cagtacccct tatgttatac tgtacggcat tattttatac tatttgttac
1381 cttgtaaagg tggcaatgaa tacatgaaaa taccagcatt atcgatacaa agtataattt
1441 tcacaaactt ggtatgcttg aaacacagat cgggatgtta ttagatgtga caaaagctaa
1501 tgatgttaag cctcgatagg accttcagtg gacttaagcc agttgaagga aacataaacg
1561 catcaaagct taagtccaag atgacataac attggaattt taaatgtatt tgctctttca
1621 gggacaaaga ccccttcatg tccctgtctg ttgctttata atggtacttt cttaggaatg
1681 aattaagttc aaatgcagag aattgccagc atataaaacg caaggatcag aaccctgagt
1741 ttgaactcag cccctgtgta cagcctccgt gtggcctctg tttaattaga tcgtgctgct
1801 atagcagttc cttctagctc agttgctttg atgtagtacc caaattttgg cctaaaagtg
1861 atttaattag taataatttt taaagatata ggatgttgaa caaagtatag cacaaagaag
1921 atgtgatttg aggattgtat aatcataatg tcctgggaac ttcttaagta aaagatcttc
1981 ttaaatggat ctcaggtctt tattttcctg tatcagccag agttgaacaa acttttgttt
2041 aaaaaaagag ccagatagta aatattctag gctttatggg ccataaagtc tcttggagct
2101 actcatttct acagttttag agcaaaagca gccatagata gataataggt aaatgaatgg
2161 gtgtggctgt gtttcaagaa aactttaaca aaagctggca gctggctaga tttggcttgt
2221 aaggtgctat atgctgaccc ctgcttatta actaaagtca gtattgcatt ctgttttgcc
2281 tgttcatatc tatgaagact tagaattctt attcatcctt tctgggattc aggtgccaca
2341 tgggcagaga aacgtggttt ctatcaaatc atctatataa aatactttat aagtgaaata
2401 ttacttgaat cctttgagat gttacaagtt tttttttccc ttgagtcatg ctaatagatg
2461 gttcaataca tgaatgagtc ccttgctgaa atgctttagg acttcagact accctgaacg
2521 ttgattactc tttatactga aataggcatt attcagtgga agagagggaa gaccaaattg
2581 atagactgga ctttattcga aaccagatga acctttttaac actggatgtt aagaaaaaaa
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2761 aatgttttca gatttttcaa tttaattttt ttgaataatt gataatcttg tttaattatt
2821 ttaactccaa aattttcata ttttcaggtt tcatgtgcaa tgacagatga aatttgtcga
2881 ctgtctgttt tggttgatga attttgttca gagtttcatc ctaatccaga tgtattaaaa
2941 atatataaaa gtgtaagtta aagtatagat aaaattattc agagacagtt tcttattatt
3001 ctataccctc atttatttca tggtttggca tttcagtgta tcagtacaaa atgaaactgt
```

FIGURE 8B

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3061 tagatctctt gtgccctctt gtaataaatg taaactgtct tgtataaaaa gtaaatagaa
3121 aatttatact tagaatgaga taaagatcat tttaggacga ggcacagtgg ctcatgcctg
3181 taatctcaac actttgggag gccgaggtgg gaggatcagt tgggccccag agttcgagac
3241 cagcctgggc aacacagtga gaactctaat ctacaaaaaa cagttaggct ggttgcggtg
3301 gctcccgcct ttaatcccag cactttggga ggctgaggcg gacggatcac gaggtcagga
3361 gttcgagacc agcctgacca acatggtgaa accctgtcta ctaaaaatac aaaaaaaaaa
3421 ttagccggtg gcaggcgcct gtaataccag ctactctgga ggctgaggcg ggagaatcgc
3481 ttgaaaccgg aaggcagaag tggcagtgag ccaagatcac accactgcac tccaacctgg
3541 gcaacaagag caaaactcta tctcaaaaaa aataaaaaat agccaagcat ggcgacacgc
3601 ttctatagtc ccagttactc aggagtctga ggcaggagga tcgcgtgagc ctaggaggtc
3661 aaggctacag tgagtcaaga tcaaagcact ccagcctagg caacaaagca agaccctgtc
3721 tcaaaaaaaa aaaaaaagtc aaattaaaaa gaccattttg gcatttactg aatattttat
3781 gtctttataa aaactacata ctttctggag aaaaaataat atggatattt accattgtta
3841 acaggaatta aataagcaca tagaggatgg tatgggaaga aatttggctg atcgatgcac
3901 cgatgaagta aacgccttag tgcttcagac ccagcaagaa attattggta atatttatgt
3961 ctacaaggtc atgtctggtt tgtttttca ttcatgactg gtgaagagct tattttcctt
4021 taggcattcc attgaaggta aaacatttac cattcttatc ttaagtgttg taattttgtt
4081 ctttctagaa aatttgaagc cattacttcc agctggtata caggataaac tacatacact
4141 gatcccttgc aagaaatttg atctcagtta taatctaaat taccacaagt tatgttcaga
4201 ttttcaagag gatattgtat ttcccttttt cccttgggct ggtcttccct tgtacatcga
4261 tttttgggcc tagaaatgct caaagggtgc tcctaggatt atcagagcct atctttcagg
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4501 aaaatgaaaa actttttgag tacggtgaca ccacaggtgg aaaattccac accagacctc
4561 aatgaggtca tagtaaaaac gcaggcacac aacacacagt ttattcagtg tcctcaaggg
4621 aaaaaagact cagcctcttc tagctgcaat atatcttttc cacgtatgcc caaattcccc
4681 cacacaagca cacctatgaa gggtactaaa atggcacatg tgcaggtcca gtgcaccaac
4741 agcattttcc ccatgatgcc ccacaagggg ccaagacctt tgtgcattac ccagtatggg
4801 cttctttact tcctgtcttt tctctggtgt aaagatactg tttaaaaaaa attttttttt
4861 gagtggggat gcgtttgaga cagagacctg cgctgtggct caggctggag tgcagtggtg
4921 caatcttggc tcactgcaac ctccacctcc cgggttcatg cgattctcct gcctcagcct
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5221 ggcatttatt tttatttata acacataaat ttaagctgtt ggaaaaactg gacaataatg
5281 taagtgagaa acttcttaca gaagaatgat gttggaatga acaccatata tgacctaaaa
5341 gaaagaacaa ctttcctaaa gttgttcttt aggactaaac tgttgaagtt ccatgctaaa
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5461 ttgatcatgc attgaaagag tggatccatc agcattgcag tgaacacgtg ccacttaagg
5521 acatgctgat catgaaataa acatctatta aaatgaactg aaaatcgaag ggaactgtga
5581 gtattcaata ggctggttag agaaatttaa ggtaagatag cattaacttt tctaaaggtt
5641 tgtggtgtta aagcatcttg atcacaaaac agcagagaaa ttcattgatg aatttgccaa
5701 gattgtctct gatgaaaatc tgactccaga acaagtctat aatgctgatg aaacatcact
5761 gttttgccat tattgaccca gaaagacact gactacagct gaggagacag cccctacaag
5821 aataaaggat gcaaagaaca gaataactgt gctgggatgt gctaatgcag caggcataaa
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5941 ttttactagt ccattattgt actaacaaaa aggcatggat ccctagggac atcttttctg
6001 attggtttca caaacatttt ataccagctt gtgtgcactt gcagggaagc taggccggat
6061 gatgactgca agattttgtt attccttgac aactattctg ctcatcgtct agctgaaatt
6121 cttattaaaa aaagtgtttc attatttcaa ccttgtgacc agggtattct acgatcaatg
6181 aagagtaaat acaaaaacct tttttgggca gcatgctagc agcagtgaac tgaggcctgg
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FIGURE 8C

6241 gtgtggaagg ttttcagaag gagtttagaa tgaaagatgc catagatgct tttgccagca
6301 caaggagttt aggatgaagg atgccatata tgccagcact tggaagacag tgactcagtt
6361 gtgtatgcct gcagcctctg cctgtcacta catttattga tgatgaggag caaattggtg
6421 actttgaagt atgtcaagtg agctccttat gcaaaaaata atatcttcag agtccatccg
6481 taagctggaa gaaggatata tcaaagaagt gtttgacatt gataatgagg tttcagttgt
6541 tcattcatta actgatggca aaatagctaa aatgcttcta aatcaaggtg attatgataa
6601 tggtgataat gaagatgatt tcattgacct cgcagaacaa ctgcctatag gcagcatggt
6661 gatgggctta ttgaagcact agagcagcat gtattcataa cagaacaaga aataggttta
6721 taaaatcaag gagagacttc aacaaaaacc attgttaaag aggcaagtag gtggtactgc
6781 aggaaacatt ttaaaaggcc attcagcaca atgtcttatt atgccgagag gacccacttc
6841 ttggtccctc aactgcttct gatgtttctt ctcacctaac aaagtactgt gtaccggaac
6901 tttttaatca aaacataaca ttgtaggtag agactgaaag cctgccattg ttgctgttta
6961 acagctgata caggtgttct ggtgatgcca ctgtgctgct tagtttgaat acgttatttt
7021 tcactgttat taatggtgtg tcttatattt ttactattaa gttcctttgt gtgaatccgt
7081 gtaagaaaat gattgcttgt cagtagtatg taaattcaat caagaatgat ggtgatgcca
7141 aacaaccata gagtgttcac atgggtggct gacatagcaa cacctgtgtt ttctgataag
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7261 tcaggctatg catataaggt atatgaagca taaatgaatt ttgtgtttgg acttgggtcc
7321 catcctcaag atagctcatt atatctatga actattcaaa aatcctaaaa aatctgaaat
7381 ctgaaacact tctggtccca agcattttgg ataagggaca ctcaacctgt agtatgctta
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7561 atacaagcat ttttttaatt ttttttatt atacttttaa gttctagggt acatgtacac
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7681 tcatcattta cattaggtat atctcctaat gctatccctc ctccttcctc ccatcccaca
7741 acaggccccg gggtgtgatg ttccccttcc tgtgtccaag tgttctcatt gttcaattcc
7801 cacctatgag tgagaacatg cagtgtttgg ttttttgtcc ttgcaatagt ttgctgagaa
7861 tgatggtttc cagcttcatc gatgtcccta caaaggacat gaactcatca ttttttatgg
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9541 ctttctggag gtttcctata aatgcaatca tatagaatgt ggccttatat gactggtttc
9601 tttcacttag tataatgttt tcaaggttta tcatcatggt atcagtgttg taggatgtat
9661 cagtacttca tttcttatta cggctgagga atattccatt atagatacaa cactatccat

FIGURE 8D

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 9721 tcaccagttg atggacatta gggtagtttg tagtttttgg ccattatgaa tactgctata
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 9841 taggttctaa tgggtataga gtagtattcg ttgtaggttt gatttgcatt tccctaatga
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 9961 aaatgtctat tcaaagcttt tacctatttt taaattattt gggtcctata gagttgcagg
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10081 tttatttttt gagacaagga cttgctatgc ccaagctgga gtatggtggt gtgatcacag
10141 ttcactgcca cctctgcttc caaggagctg ggaccacagg catgtgccac cacacctagc
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10261 actcctggtc tcaagctatc ctcctgccat ggcctcccag agtgctggga ctacaggcat
10321 aagtcactgc acccagcccc aaatatattt ctcttgcctg tggctgtgtg ttttgaagtt
10381 taagaggttt ttattttgaa gtcctttttt tttttttttt tttttttgag actgagtttc
10441 actctgttgt ccagactgga gtgcagtggt gcaatctcgg ctcactgcaa gctctgcctc
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10561 cacctcgctg ggctaatttt tttttttttat gtatttttat tagagacagg gtttcactgt
10621 gttagccagg atggtctcga tctcctgacc tcgagatcca tccacctcgg cctcccaaag
10681 tgctgggatt acaggcgtga gccaccgcgc ccggccgaag tccttttttt ttttttttagt
10741 ttatcatttt ttgttgttgt ttgtttcaag gattgtgctt ttgttgttag tttttttaaa
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10861 attatgaaaa tttattgttc atgatatcat tggtccatat tgtagataag aatttccaaa
10921 ttatttttat tttctacata tatttgtggt gtactcatca tttagggtaa tttttccctc
10981 atagatgtgc tactataatt ttagagtttt ataaagataa ctttatagtt gtttaagtgt
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11461 aagcattttc acatcacagc acacaaagaa atatttgtac gttgaggtaa taagaaaacc
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11641 tatgcttgtt ttggtttttg aaaggtctga cctacaatat ttaacattgt tttatttttg
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11761 atcccttta agcctgattg atttctggta acttcaggtt ttctcatgga gaaggaatat
11821 acatttttag aaaatgtatc taactcaaag atccataggg aactaaaatg cttttaaatg
11881 tactctccaa agtggtgttt ttccttctca gactaagcta tgactttatc ttacagattt
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12181 tatgacatac atgcagaaaa agcacaaaat aagtgtattg ctcaaagaat tatcacaaaa
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12361 acattagaaa attggtagat aggaactctc aagaactctg gaggggttta aaaagatagt
12421 tcttaatttt ttttcttttt tttcagagat agggtctctg tcgcccaggc tggagggcag
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12661 tcaagtaatc ctccctcctc aagcctcctg agtagctgag attataggca tgagccacca
12721 tgcccaactc aaaagatctt cagcagacct attctaaatt tatgtacctg gctgggcaag
12781 gtggctcacg cctataatcc cagcacattg ggaggctgag gcaggcggat cacttgaggt
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13021 cagcctgggc gacagagtga gactctgtca cacatacaaa aaaaattaag cactggatat
13081 agatttattt ttctattctt tgtctttttc tccttagaaa gtgaaacaga aaaaaaacaa
13141 aataaaataa cttctaattg attaagaatt caggttattt gtgttcttat taataggggt
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FIGURE 8E

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13201 tattctataa catttaggaa tgcatcaaaa ttcatgatca gatatcactt gccaagaatg
13261 ggggcttcat cagatccgga atagaattta tctaaaagtg atcaagacat gcagacttat
13321 aaaaagctat gaacatcctg tctgtataac aacttggcca gcaacattcc tggcgcaaag
13381 ggctaaggct ccttcaagcc ttgagaataa gacacttaaa agaataagcc caagctcctc
13441 ctgagcgagg aggccgaata ttgtcagtag aagcatggac ttttggatgt gatctgttct
13501 ggagccccgg acctagccct tgttactttg tgatttttgc acaagttcct cggactctct
13561 ggtcttctgt gtcctcatct gctgaatgcg caaaaagttc ctacctcctc aagttctgtg
13621 tctgaaggac attatgttct catagcactg agcacaatcc ctggcacatg gttactcagg
13681 gcacccaagt tatcattatg tgtctaggga aagttgggtt gggcatgcag ttgttgaatt
13741 ctcttctttc tgggtgagcg ctgcctctca gcagctgatg gggaatcct tgcattattg
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13861 ttggatctca aaggcagatc tttttttgtt ttgtttttgct ttcttgagat ggagtttcgc
13921 tctcgttgcc caggctggag tgcaatggca cgatctgatc gtggctcact gcaatctctg
13981 cctcctgggt tcaagtgatt ctcctgcgtc agcctcccaa gtatctggga tgacaggcat
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14101 aggctggtct cgaactcctg acttcaggag atctacctgc gtcagcctcc taaagtgctg
14161 ggattacggt ggtgagccac cacacccggc ttaaaggcag atcttaaaag cacattaaat
14221 catctgctct aactcccaca ttgcacagag aaataagctg agttccaagg aggcagcata
14281 acctgagact agaaatggtg cttaggttcc taagcccagg cctccaaggt ctatttactg
14341 tataatgtga gctgatgtct cccaaagtat aaatgtaggg tcacctgtgt taggatcata
14401 tgaagggctt tttaaaatgc aagttcttgg gtcctttacc ctaattgttt tatcagaatc
14461 tctagagatg ggacctggga atctgtattt aacagaggat cacacctgag ttcaagaacc
14521 actcatgtag tagaacaatt acctcaactt aaaatatgaa atgtatctgt agcaagtgcc
14581 acctggtaaa gacttgatca cagtggattt caaacaagac aaagtattga gggctgttga
14641 actgtcaaag aatttcagct attatttcta ttagtttctg cctcactatc catcgagttg
14701 tttgtatgcc aggataggcc aagttctctg gctcttgtga gcttgtgtaa gtcagtgtag
14761 ctctctgctc ttgaaaaagc ccaaaaagtg aattaacatt tgtatagact tagaactgta
14821 actagctcat aaatagtagc cactagtatt atcactcaga gcaggaaaag catctgcaca
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15061 agatcagatg tgactttact tttgacagtt tgaattcttg ctcacatcag caaaaattct
15121 ccatattgga ataactgtcc agttaggggt ttacttattc tcctacgaac aaatatagat
15181 agtcacgcaa agaacatagg ctgttgtgta aattttaggt tgttgtcaat gatttgtgca
15241 tctctaaatt ggaaaacaca gacatagttt tcatgaacat ggagaatttc agctacaaaa
15301 taattcttag ccataatagg tattgtatat ttaattgaga gaatgtgaaa aacaatgagg
15361 aagtagtttc tccagtatgg tgaaaggcaa agagggttct tttttttccc ctaagtaaag
15421 catctactaa atgcaaaaga aatgattgtg gactctggaa tctggaatcc acgatgctag
15481 cactttgcag taatagcctc tttcatatat agatctcaca acagtttgta gacactaagt
15541 ttttccatgc tcctgcaact cacactgagt ttttatttac tccctttttta ctctggaaaa
15601 gcagggcagg aagtttttaa agggttcctt gcagttacaa agctagaatt tgaacccagt
15661 gtcaaagcct ctgggtcaga ttgggagggg gcccacgttg tgaacttgac agttaattca
15721 tggttgctgt tttatggagc aggaagtgac ctttagtgac tcagaggaag gaataagctg
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15961 cttggctttc aggccaagag tggagtgagt gtggtacttg gtggacagag aggccagttg
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16081 tctcacttat tgtccatgag tattttcttt taaatggaac cggaatcata agataagaat
16141 gaccaggagg ctctggtaaa ggcaggtgca agttttgtgt gacaagatcc tcatggacta
16201 aaaaatgatc atatttctga ggttgaatca aatacataaa agcagcagca aatcacacac
16261 ttcttcgaag agagttacca agttgcaggg gaaatttatt agcacaaata agtctgaaaa
16321 ggaaaagcta tccatatata caaggttaag ttggaagaag gaggcagaaa gaagaaattc
16381 cagttgttat cattattttt tattccaaat tgcatctgtt cactaaaata cttcggactg
16441 ggtgcagtgg cttacaccta taatcccagc actttgggag actaggcggg cagatcactt
16501 gaggtcaggc attcgagacc agcctggtca acatgatgaa accctgtctt tactaaaaat
16561 acaaaaatta gccagtcgtg gtggcacatg cctgtaatcc cagctactcg ggaggctgag
16621 gcaggagaat cgcttgaacc tgggaggtgg aggttgcagt gagccaagat tgagccagtg
```

FIGURE 8F

```
16681 cactccggcc tgggtgacag agggagactc catctcaaaa aaacccaaac aaatatttta
16741 taaaatagaa atagaaaaat aatacaaatg aaaagtctat taagtatata tttttattaa
16801 gcaatattaa aatgactgca agtaagagtt tatatagcta tatgtacatg gatatttata
16861 gatgagatta cgcttacatt cttgcccaca ccatcttgga aaatgttaag ataatatcgc
16921 cttgactgaa aaacatacag caaacatggt ctctttggca ttctgtcatc cacatctaca
16981 ggtgcctgta gcaatgtgtg gtactataat ataatggtaa ttgatgttcc taatttggga
17041 gtgtggaaag atccccaaat gtcttttaag tcatcagaga aagataaaat aatatttgat
17101 acagcttttc ttaaaatttg agataatttt aatggcgagt tattttatgg ccctttgtat
17161 cttgaaaaat tgggaaatca catatggttt aaaagcgaat tatcttaatt ggaacatgcc
17221 attaactaga aaacccatta tttcagcttg cactctaaca gacaatacgt ggaaaaggaa
17281 acgcggccag ggcaaaacat ttcctcttct tataaacctt gaactgagta cgtccctcac
17341 caattataga gggtcccctt gggcctcaga actttccaca agcgttgagg tctctatggc
17401 gatgctcccg gctgccgagg cggaaacaca ggtgatgagg tggcggcaag cacagtgcaa
17461 agagagagaa gcagcttcgg ctgcagcaaa ccacgcaggt ccttcttgat catctagaac
17521 tgaccgctcc gccttgccag gagtctgcag aaccacgtgg ctagcctgcc tgaagttctc
17581 acctctccag gaaggcgggg ggcttctaat ggctgcagct gcgctggggg ctgggggctc
17641 ccgctgggac tccacttccg tggatgtcta agcttcacct ttcttgcgcc cgcaggggca
17701 tgactcaggt gaaagggagc cattttctca gacccctggc ctcatgcagc ccttcagcat
17761 ccccgtgcaa atcacacttc agggcagccg gaggcgccag gggaggtaag tcacttccgg
17821 aagctctgcc ggtagtggga atctggctga acaagcagtt gcaagaagag gggacatctc
17881 gagcttgggg agtgagtgtt tcctttttct ctgaggatgc ccacttgcca tgcctcccag
17941 ggtacccagc aggcttcccc agtagcactc acatcacggg gctgcagcct ttcctgttgg
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18061 ccccagcact gatcatagcc cagcccatag ttggtgctca agaatgatct gttggttgaa
18121 tgaggaatga agaaattaga ccagcatttg gtcccattgg tgaagccctg gagtcacagc
18181 ccttggattc aaacccagct caccacttaa tcagccatat gactgggcag gtcccattgg
18241 tgaagccctg gagtcacagc ccttggattc aaacccagct caccacttaa tcagccatat
18301 gactgggcaa gtcacttaac ttctctgttt gcctcatctc cttatctgtg aaatgcagat
18361 agtaagagcc cctgcgtgtc tcagcacagt ataccacatc ctctctaaac tatactgtta
18421 taccgggtat acgctctaca cctccctaaa ctctagcctt ttagctattg ttattaccca
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18541 agggaaatga aaagcaaaga agctgaaagc ctctagtgac ctgcacccct tttgcctgcc
18601 ctagggttgg ggcatggggc agtcaatgtg ggaaccaaaa tggagagaag agtgatgcct
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18721 gtaatcccag cactttggga ggccgaggtg agtggatcac ctgaggtcag gagttcgaga
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18961 caagagccaa actctgtctc aaacaaagaa aaacctgttg ggaggggtac atttcagaac
19021 caggttcact cactatctgg aaatatgcat gatttattat tggctctagt ggagttggga
19081 gctgagaatt ggaaaacatt aaagatggta atggtcatca tgttactcac ttccattatc
19141 acttaactgc actcaggggt atttgagagg gagcatgagg agagtgagat acaggagttt
19201 ggataagatg ggggttcagg gaagaaggac cagacagact acagaggaaa gaaaggtgtt
19261 cttctcgcta gacatgaacc aattttttg tgaacagaca attaaaatga attactttat
19321 ggcaaaagat caaatgacaa acatgcaagc aaacaagttt tagtgtccca tacgtcacac
19381 aattaactag atataaaggc agttgtgttg tcatcaaagc aaactactgt atcccatttt
19441 catttctgaa atgcacaact gaattattgc tatttcctct tgctgaactt gatgaactat
19501 gttgacttaa ccttatttgc tgtttcaaaa taagttgtta aataatgttg taattaaaaa
19561 atagaagagt aaaaataatt accaagggtc tcctctgtaa ccaaaacgaa ttacagggaa
19621 atattaatat agtgtacttc atgtttagac attcatttca catacacccg tgaaatgaat
19681 gtcagatgaa ttatgaagtt ttgatgagcc acagcatgtt tctaaggaat acttcttgca
19741 aaagtttcag tgctggaggg agaggctgct ggcttctgtg gggatcacac ccaggtgagt
19801 gtgttcaggc tgtttgtaat tgagtttgcc tcaggctaag ccagaagctg cctgtagcca
19861 tgtgtcgtac ttgggctggg tgggaaagtc agtcctatct gcagtgaaga gaatagaagg
19921 tggtggatat tgccttgatt atgaataaaa cagctcaagg taatacactg gttagaagcg
19981 gacatgtatt actggcagaa aaaggaatca atatgctttt tatccatctt cctgactaga
20041 aagcaaacta gatcatactt aagtgctttg aggtccttgg atgaaagatg cttgtaaata
20101 caacaaagtt aattacaagg ctgtttatgg tctgagaaaa ctggaaacaa cctaataata
```

FIGURE 8G

20161 ttcaataata aggaaatggt taaggaaata tggcatatct aattgatgga acattatgta
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20281 aaagataaga ttaacaaaaa aatctcaaat catatctaat gtgatctcat tctgtttaaa
20341 acaatatagg ccaggtgcgg tggattatgc ctataatctc agcactttgg gaggccgagg
20401 caggtggatc acctgaggtc aagatttcga gactagcctg gccaacatgg tgaaaacccg
20461 tttctactaa aaacaaaaaa attagctggg cgtggtagcc ggcgcctata atcccagcta
20521 cttgggaggc tgaggcagga gaattgcttg aacccgggaa gcagaggttg tagtgagctg
20581 agatcatgcc actgcactgc agcctggatg acagagtgag actccatctc aaaaaaaaaa
20641 aaaaaaaaaa aaagaaaaga aaaagaaaaa caaaacagaa acaaaaaaca aaaaacaaaa
20701 aacctaatat agagcaggag gggattaacc cagcaaacca agtgcacaat cattaccttt
20761 aagtgttgtg ggtgagtttt tgttctcttc tgtacatttt tttttgtatt tttcaagttt
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22321 gagtgtataa aaatacatca taatctgata gtggattttg tctatttctt cttgtagttt
22381 aatcagtgaa acaatgctat caggtaccta caaattagca ttgttacatt ttccgtgtga
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23461 ccatcgacac cgaggcgtcc atcagggaaa tcaaggactc tctagcagaa gttgaagaga
23521 aatgtaagaa ggctatgttt tccaatgctc agttagacaa tgaaaacaca aacttcattt
23581 accaagttga caccctgaaa gatatgttgc tggagattga agaacagctg gctgaatata

FIGURE 8H

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23641 ggcggcagta cgaagagaaa aacaaataat ttgaaaggga aaaacacgcc cacagtatac
23701 tgcagtttca gtttgctgaa gtcaaggagg ccctgaagca aacagaggaa atgctcgaga
23761 aacatggaat aatcctaaat tcagaaatag ttaccaatgg agagacttcc gacactctca
23821 gtaatgtttg ataccaagat cctaccaaga tgacgaaaga agagttaaat gccctcaagt
23881 cgacagggga tgggacccta ggaaagccag tgaggtggag gtgaagaatg aaatcgtggc
23941 gaatgtgggg aaaagagaaa tcttgcacaa tactgagaaa gaacaacaca cagaggacac
24001 agtgaaggat tgtgtggaca tagaggtatt cactgctggt gagaataccg aggaccagaa
24061 atcctctgaa gacactgccc cattcctagg aaccttagca ggtgctacct atgaggaaca
24121 ggttcaaagc caaattcttg agagcgcttc tctccctgaa aacacagcac aggttgagtc
24181 aaatgaggtc atgggtgcac cagatgacag gaccagaact ccccttgagc catccaactg
24241 ttggagtgac ttagatggtg ggagccacac agagaatgtg ggagaggcag cggtgactca
24301 ggttggagag caggcagaca cagtggcctc atgtccttta gggcatagtg atgacacagt
24361 ttatcatgat gacagatgta tggtagaggt cccccaacag ttagagacaa gcatagggca
24421 tagtttagag aaagaattca ccaaccagga agcagctgag cccaaggagg ttccagtgca
24481 gagtacagaa gcaggtaggg atcacaacga agaagagggt gaagaaaaag gattaaggga
24541 tgagaaacca atcaagacag aagttcctgg ttctccagca ggaactgaga gcaagggtca
24601 ggaggcgaca ggtccaagta cagtagacac tcaaagtgaa ccctcagata tgaaagagcc
24661 agatgaagaa aagaatgacc aacagggaga ggcattggac tcattgcaga agagaaagaa
24721 caagaaaaag aaaaagaaga ggaaaaaatc cccagtaccc atagaaaccc ttaaagatgt
24781 ttaaaaagag ttaacttatc agaacacaga tttaagtgaa attaaggaag aagagcaggt
24841 aaagtctact gacagaaagt cagcagtgga agcccaaaac gaggtgactg aaaatccaaa
24901 acagaaaatt gcagcagaaa gcagtgaaaa tgttgattgt ccagagaatc ctaaaatgaa
24961 gttggatgga aaacttgacc aagaaggcaa tgatgtaaaa acagcagctg aggaggtact
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25141 ttaaagcaat cctgaagaac cagagagcga agatgcagat cactgcaccg tacccaaaaa
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25381 taccagagga gggaacgaga agggcaaaag caaagaagac cgtaccatgt cctaagctga
25441 ggcaggcggc aggcgtggtg cacaggaagt ctgagtgtga ggggctcttt tctctccact
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25561 atctactgct ttaagttata gactgttact tgtagatttc catgtaatca ttgaggttat
25621 cacccagatt agaaagacat atttgttatc agtgtacatt ctaattgaga gcatatatcc
25681 agtagtatca aacaataatg tctactgttt atagtccact taataaaaat agaagcattt
25741 accatttgcc ttaggctgat aggaatgtga atattcttga ccaaatatat cagcatctaa
25801 ttgaaatgac caaatagcat tcttagactt ctgtattatg aatataattg atatttaaat
25861 taatgtcttg ttcatatatg tgtactttca tatttgattt taaaatatac attataacct
25921 gtatggtatt ttatttaaag gagataaacc gccaaatagc aaataggtca ctgaaaagat
25981 ttgcacctta gaacaataat cattttaagg ataacaagta aaggtctgaa agcatgaggg
26041 gctttatttg ccttcacctc atataagtct ttgattttga accaatgctt ttggatctca
26101 ttgttgatga tacttgaatt tactttgtag gagattttaa cttcatgctg atgatgtatc
26161 aaattcattt tatacaaagt ttaaagattt tttctggaag tgatacatgt caaattacat
26221 ttcctactgc agtatttgag cagggacagt catttttaa atgtttttgg ccgggtgtgg
26281 tggctcacgc ctgtaatctc agcacgttgg gaggccaagg cgggtggatc acctgaggtc
26341 agaagttcaa ggccagcctg gccaacatgg tgaaaccctg tctctacgaa aaatacaaaa
26401 aattggccag gcgtggtggt gggcgcctgt aatcccagcc actccggagg ctgaggcagg
26461 agaatcgctt gaacctgcga ggcggagatt gcagtcagcc aagatcaagc cattgtactc
26521 cagcctggac aacgagcgaa actctgtcta aaaaacacac acacacacac acacacacac
26581 aaaacaatgt tttcatgcct gtaaccctag cacattggga agccaagttt ggaggatcgc
26641 ttgaggccag gagttcaagg ctgcagtgag ctatgattgc accactatac tctagcctgg
26701 gagacagagt gagaccctgt ctctaaaaaa aagaaaaagt ttttgaacct taaaattact
26761 tctctttgtt tgaatttcta atcatcattc aaaagaacag ttaaaaaagg ttacttgttc
26821 ttgtgcaact acaaattaga ctggagtagg atattttaaa gagctgaatc acttttggta
26881 ttttgttata aatgttttca tttgttatgt cccagtatat tcttattgga aaattcttgt
26941 tttgatctgc ctgaagaaaa tatctgtttt ctatataaag aaacatttaa aaataattgt
27001 aaagttagat ttaattgtaa aatataaaat cacaaaggaa tgtaccttat gaatgttgac
27061 attttatgaa attatgtaga ttcatattac tgttacaaga tagaattgaa tgcaaaaaga
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FIGURE 8I

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27121 ccaaatcctc attaaaattt gaggaaaaca taagtgttat tatgtaattg aaataaaaac
27181 attttatagt tgtaaaaaaa attgcaagtg gaaccatctt aagttggggg acatctatat
27241 gtatttaaat ctagtctgac aatctttata tttgaaaaac agttttttta gagatagggt
27301 ctcacctatc actgaggctg gagtgcagtg gcacaatcaa gcttattgca gcctcaaaca
27361 cctgggctca agcaatcctc ctggctcagc ctcctgagta gctaggacta taggtgtgcc
27421 actacaactg gatggtgttt taatttttat tgtgtagaga cagggtcttg ctatgttgcc
27481 caggctggtc tcgaactcct gggttcaagt gattctccca cgttggtttc ccaaagtgtt
27541 gggactatag gcatgagcac cacagcctgc cctactctct atcttttaac tggatcattt
27601 actccattta gttttattgt aattactgat atactgatgc aataacatta ttctatcatg
27661 ttattctgtg ctatttgtcc tgacttnttc atgagttttt ccctcatctt tattgccttt
27721 tttggattga ttttttccct ttccattctt tgtttctcta ctagtttgga atttctggag
27781 tatcacctaa aagagtagag aaaggtgata tttctattta gcatgcatat ttgaactttt
27841 caacatgaaa ttaatgtctt tatttttccc catggaaaaa ttatcactgt tactccctct
27901 aaattatatt ctcttgtcat gtatttatct tttcttttaa ccccacaaga cataatagta
27961 ataagataat tactattatt attattttgt agaattaata tctttttttt tctttgagat
28021 ggagtctttc tctgttgccc aggctggagt gcagtggtgt gatcttggct tactgcaacc
28081 tctgcctcct gggtttaggt gattctcctg tttcagcctt ccgagtagct gggattacag
28141 gtgtgtgcca ccacgtccag ctaatttttg tatttttgt agagatgggg tttcgccatg
28201 ttgggcagtc tggtctggaa ctcctgacgt caggtgatct gcccacctgg gcctcccaaa
28261 gtgttgggat tacaggcgtg agccactgtg tccagcctag agttaatatc tttacttacc
28321 tgtttacttc tgtatttgct cttcatttct ggttaaatat cagctgttat ctggattttt
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28501 ttttgtagac atagaattct gtggttactt tttctcagta tattgataat attcttgcct
28561 tatggcttct agtcttgtta cagagaagta agctctcagc caaattgtca ttactttgaa
28621 gttaattgtc tttttctttg gcgactgtta agatttcctc ttgtctttgt agctgtgcaa
28681 tatatctgtg atgtgtttaa gtgtggttcc tctttatttt atcccatggg cttctggagt
28741 ctgggaactg gtcttcaatc agttctagaa tttgactatc tttttaaaat attgcttctg
28801 acttatgtct cttttcttct ggaattctga atagatatta tgttacacta tttaatctat
28861 ctttcatgtc tctgaacctc tctttaatac tttccattta aaaatctctc tgtattatac
28921 tctggctatt tttgcagatc cagctctgtg ttcactaatt atcttctcag atgtatctaa
28981 ttgagtgtta tgtctgttca ttaaattttt actttcaatt attttatttt tgatttctat
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29221 tcctgtgtgt tttatgactt ttattttatt ttttattatg tatttattta ttcatttttt
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29401 gattacaggt gcctgccact acacctggct aatttttgta tttttaatag agacagggct
29461 tagccatgtt ggccgggctc gtctcaaact cctgacctca ggtgatccac ctgccttagc
29521 ctctgaaagt gctgggatta tagccatgaa ctacctcgcc tggctgttgt gtgttttatg
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29641 ttttatttga gacgagtctt gctctgtcgc taggctggag tgcagtggcg caatcttggc
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29761 ggcacgtgcc accacgccca gctaattttt tgtgttttta gtagagatag ggtttctcac
29821 catgtaggcc aggatggtct cgatctcctg acttcatgat tcgcccacct cgacctccaa
29881 aagtgctggg attacaggcg tgagccattg cgcctggcct atagaattct tttaaggcct
29941 aagttaaatg tgtgtgccta cagagaacat acgtatttaa atttgccagg ttgcagaggg
30001 cactacctac ttaaacaaca ttacacgaaa ttcttagctt gagatttttg tattacccag
30061 gtagtatgaa ttcaggctgt aaactccctt gaggatcccc ttgaggatta tctaaaattt
30121 caggggagat tgtagttttc ctcttttagt cagtgttaag gtttgagaaa ggcattttcc
30181 ttgccatttc ctatggagtg gtacggtgtg ggtgaaggga gaggggctat ttccagttca
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30361 aacaaaagct caagttcacc aagttccgca aatgccctca agttaaaact tgacttctgt
30421 ccaccttcct ttctgggttc ctactttcac atagttttg tcttttgagt atttccaatt
30481 ctttttaagc tttggccaga agtttagtt gtctgtagtt agagtgggtg tctagtgtac
30541 cataccactg aaacagaagc ctgttacttt tacaataata aaaacatatc tatgtgggct
```

FIGURE 8J

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30601 ttttaaaaaa atttttaaat ttatttattt actttttttt ttgagatgga gtctcactct
30661 gtcgcccagg ctggagtgca gtggcatgat cttggctcac tgcaacctct gccacccggg
30721 ttcaagcgat tttcctgcct cagcctcctg agaagctgtg attacaggcg catgccaccg
30781 tgcctggcta aattttgtat ttttaggaga gacaggtttc accatgttgg tcaggctggt
30841 ctcaaactcc tgagctcagg tgatctgccc accttggcct cccaaagtgc tgggattaca
30901 agcatgagcc actgcacctg gccaaaccta tctatgtttt agttttttat gattatttta
30961 tattatccct tccccacaca tgaaataact tctttccaag tgacttatga agttgtcaac
31021 ttttcataaa gccttggaac aaagtgggca gaaaaattat aaataaaaag tcttgtctag
31081 gacagtacag gtctgtctta ttcctaaatt agatcagaat cgggttatgc cggttttta
31141 taacatacca ttataattgg gtatgttaaa gaatgtatta gagatgcatt agaagagcga
31201 cctcattata agcctcttca cccatggatt ccaaggatat cttacaataa acctctggga
31261 taccttacgc tacagagcaa ctaaagtcca gctttagagc acaagggaaa atgcaggata
31321 ttgggtcctg gaaactacaa aaaccatcaa actctacttt agggctaaaa ctttctttta
31381 atcaatctgt catatttatt gataaattag aaaatgtggc tgggtgcagt ggctcacacc
31441 tgtaatccta gcagtttggg aggccgaggt gggtggatca cgaggtcagg agatggagac
31501 cattctggct aacacagtga aaccсgtctc tactaaaaat acaaaaatta gccgggcgtg
31561 gtggtgggcg cctgtagtcc cagctactca ggaggcggag gcaggagaat ggcttgaacc
31621 cgggagacgg aggttgcagt gagccgagat tgccccactg cactccagcc tgggtgacag
31681 agggagactc catctcaaaa aaaaaaaaaa aaaagtacag gttcaaattt cattaaattt
31741 tttttccatg ccccttctct ggtggtagga tgatagggga tgagtaaaat atcaaagaca
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32581 cctgtaatcc cagctactag ggaggctgag gcaggagaat cgcttgaacc tgggaggcgg
32641 aggaggttgc agtgaactga gatcgtaccc agcctgggca acagtgcgag gttccgtctc
32701 aaaaaaaacc aaaaaacaca aaaacaaaaa acgacagaga aggccaaaca aaacacatct
32761 gtgggctgga tgccgccatg cccaccggtt tgcgaccttt gtgttggact cttctgttca
32821 ccagacaccc tgccctgcga gaatgtatct catcctttgc tggagcaggt ttgcaggcac
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33001 ctgggcagtg gccagaaatt ctggtggtgg atatgctgcc tttccaacag gtgaatatga
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33481 ctgcagtatc tccttctccc tttccccaaa ggacagcctt tcctgcctca gggaagaaga
33541 gagagacaga ctacagtgat ggagacccac tagatgtgca caagaggctg ccatccagtg
33601 ctggagagga ccgagccgtg atgctggggt ttgccatgat gggcttctca gtcctaatgt
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33841 tcttccgctg tcagtgctgg gggcagctgg gtgtggagga agaggtggcg agaaggtcaa
33901 agatatttcc ctgcactgcc tcctcccatc tcataatcta tatatacctc caaacacgta
33961 atccacaaat tatagtttct tcatttaggt cataaatcct ccccttaaaa tgttggtatt
34021 ccaagagaga gttaaacctt gtatgtgagc acaataaaag ttttttgagt ctgaattttt
```

FIGURE 8K

```
34081 tgagtttgac agtgtctacc tggcacatag tagttgctca atacatatta gtttccttcc
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34201 gtttgaactt cgggaatcca cttataaatg gattttcttc tgtgcctgcc acccctgaga
34261 cagtaagatc aatccctcct ctttctcctc ctcctcagcc tactcaacat gaagaggaca
34321 gggagaggac ctttatgatg atccacttcc atttagtaaa tagtaaacat gttttctctt
34381 ccttatgatt ttttttcttc aatttttgtt ttaagttccg ggtacatgt acagaatgtg
34441 caggtttgtt acaaaggtaa acgtgtgcca tggtggtttg cagcacagat caacccatca
34501 cctgggtatt aagcccagca tgcattagct attcttcctg atgctctccc tcccccctact
34561 ccactgagag gccccagtgt gtgttgctcc cctctaggtg tccatgtgtt ctcatcattc
34621 agctcccact tttaagtgag aacattcagt gtttggttct ctgtttctgc attagtttgc
34681 tgaggataat ggcttccagc ttcatttcat ctatgtcccc gcaaatgaca tgatcttgtt
34741 cttccttatg atttctttc cttttctttt ttctgagacg gagtcttgct ctgtcaccca
34801 ggctgaagtg cagtggcacg atcttggctc actgcaacct ctgcctcccg aattcaagca
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35161 ctttagttgc tacttattaa gtatatgctg tatgctcaat acatactata ttttatccta
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35401 gggaatgggg gttctgatta gggaagggga ggggctattt aattttgtag gatggcccac
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35521 tttggacatt gctttatttt tggacagccc tttttttttt ggacagccaa agagcaacag
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35641 aaaaggaaga gagtggccta cttctgttat ttatgattca tctcctgcca ttcctaggtt
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36541 tagaaactgc cggaaatcgt cgtggtattc actccagagc gatgaaaacg tttcagtttg
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37441 tgaaacaatt atcctgagaa taaatgcctt ggcctttata tggaaatgtg gaactgagtg
37501 gatatgctgt ttttgtctgt taaacagaga agctggctgt tatccactga gaagcgaacg
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FIGURE 8L

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37561 aaacagtcgg gaaaatctcc cattatcgta gagatccgca ttattaatct caggagcctg
37621 tgtagcgttt ataggaagta gtgttctgtc atgatgcctg caagcggtaa cgaaaacgat
37681 ttgaatatgc cttcaggaac aatagaaatc ttcgtgcggt gttacgttga agtggagcgg
37741 attatgtcag caatggacag aacaacctaa tgaacacaga accatgatgt ggtctgtcct
37801 tttacagcca gtagtgctcg ccgcagttga gcgacagggc gaagccctcg agtgagcgag
37861 gaagcaccag ggaacagcac ttatatattc tgcttacaca cgatgcctga aaaaacttcc
37921 cttggggtta tccacttatc cacggggata tttttataat tatttttttt atagttttta
37981 gatcttcttt tttagagcgc cttgtaggcc tttatccatg ctggttctag agaaggtgtt
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38101 aattgccctt aaccctgtga caaattgccc tcagaagaag ctgttttttc acaaagttat
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38281 aatcgtccag tcaaacgacc tcactgaggc ggcatatagt ctctcccggg atcaaaaacg
38341 tatgctgtat ctgttcgttg accagatcag aaaatctgat ggcaccctac aggaacatga
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39061 agttttgctg tttccttcag cctgcatgga ttttctcata ctttttgaac tgtaattttt
39121 aaggaagcca aatttgaggg cagtttgtca cagttgattt ccttctcttt cccttcgtca
39181 tgtgacctga tatcgggggt tagttcgtca tcattgatga gggttgatta tcacagttta
39241 ttactctgaa ttggctatcc gcgtgtgtac ctctacctgg agtttttccc acggtggata
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40921 atgctgtttc atttaatcaa tgtttattca tggcaaattt tatttttat tatttttatt
40981 ttttatttta atttaagtgg gtacatagta gttttatgta tttatgggtt acgtgagata
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FIGURE 8M

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41041 ttttgataca ggcatgcaat aatcacatca gggtaaatgg ggtgtccatc atctcaagta
41101 tttatccttg tgtcacaaac aatccaatta tactcttagt tgtttaaaaa tgcggggagt
41161 ggagggcaag tggagggaga gcattaggac aaatacctaa tgcatgcggg gcttaaaacc
41221 tatgtgatgg gttgacaggt gcagcaaacc accatggcac atgtatacct atgtaacaaa
41281 cctgcacgtt ctgcacatgt atcccgtaat gtaaagtaaa ataaaatagc ataaaatcaa
41341 ataaaggaat cactaatgaa acctacattt gcaaaataaa ttataaatat tgaaaagaaa
41401 taaaaagtac aaaaaattat ttttgtctat agtcactcta ttgtgctatc aaatactagt
41461 cttattcatt ctttttgttt tttgtaccca ctaaccatcc cactccccgc cctacacccc
41521 ccaacaaccc gccccatact cttccaagcc tctggtaacc gtccttctac tatctccatg
41581 agtcgaattg ttttaatttt taacttccac aaataagtga gaatatgtga agtttgtctt
41641 tctgtacctg gcttaaattt cacttaacat aatgacctcc agttccatcc atgttattgc
41701 aaatgactgg acctcatttt cttaatggct gaatagtact ccattgtgta tatgtaccac
41761 attaaaaaaa atcaatttgt tagttgatgg attgctttca aatcttggct attgcgaata
41821 gtgctgcaat agctaagatt tggatatctt tgggagtgta gatatctttg ggatatcttt
41881 taatcttttc ttttcttttt gagacagagt ctcgctctgt tgcccaggct ggagtgtagt
41941 ggcgcaatct tggctcactg caacctctgc ctcccgggtt caaacaattc tcctgcttca
42001 gcctcctgag tagcttggat tacaggcact caccaccatg cccagataat ttttgtattt
42061 ttagtagaga cggggtttca ccatgttggc caggctggtc tcaaactcct gacctcagat
42121 gatccaccca tctcggcctc ccaaagtgct gggattacag gcgtgagcca cctcgcccag
42181 cctgattttt tttcttttga gtatatacct acctctattt tcagtttttt gaggcacctc
42241 caaactgttc tccatagtga ttgtactaat ttacattccc actaacagtg tacaagggtt
42301 cccttttctc cctattcttg ctagcatttg ttattgcttg acttttggat aaaagccatt
42361 ttaactgggg tgagatgata tgtcattgta gttttgattt gcatttctct gatgatcaat
42421 gacaatgcct ttttgtatgc ttgtttccca cttgtatgtc ttcctttgag aaatgtctat
42481 gcagatcttt tgcccattta aaaaactgga ttattatatt ttttcctata gagttgtttg
42541 agctcctttt atattctgat tattaacccc ttgtcagatg ggtagtttgc aattattttc
42601 taccactctg tggtttgtct cttcactttg ttgattcttt cttttgctat gcagaaagtt
42661 tttaatttga tatgatccta tgtgttcaca tttgctttgg ttgcctgtgc ttatgaggtt
42721 ttactcaagg aatctttgcc tactctggga cttactgttt tcttttacta gtttcatagt
42781 tcgaggtctt aaatttcagt ctttaatcca ttttgatttg atttttgtat aaggctagag
42841 ataacaggtc tagttcactc ttttgcatat ggatatccag ttttcccagc atcatttatt
42901 gaagagactg tcgtttcccc aatttatgct cttggtaact ttgtcaaaaa tgaattcact
42961 gcagatgtat gcatttacct ctgggttctc tattctgttc cactgatgta tgtgtctgtt
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43441 gattgttcac tgttagcata tagaaatgct actgatgttt gtatgttgaa tttgtatcct
43501 gcaactttac tgagtttgtt tatcagttct tatagttttc ttgatggtat ctttagtatt
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43621 gatgcccttt atttctttct cttgtatgat tgctcaagct aggactttca gtattatgtt
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43741 caatttttcc ccattcagta tgatactaac tctggatctg ttgtatatgc tttttattat
43801 gttgaggtat gttccttctg taaccagttt tgtgaggttt ttgttatgaa gggctgttga
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43921 tgttgatatg atgtattaca ttaattgatt tgcatatgtt gaactatctt tgcatcccta
43981 ggctaaatct cacttcgtca taatgaatga tgagtgattg agttagtgaa tattgatgag
44041 ttatcgaata ttgagtttga tttggtagta tttgagaatt tttgcatcaa tattcatcag
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44281 ggtgtcactc tgtcaccaca ggctggagta cagtggtgtg atcttggctt accatagcct
44341 ctgcctcgcc tcccaggctc aagcgattct ctaataatat ttgctttata tatctgggtg
44401 ctccagtgtt tggtgcatat atatttatca aattgttata tcctcttgct gaattgaccc
44461 ctttatcatt atatagtgac cttcttgttc tcttcttata gtttttgtct tgaaatctat
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FIGURE 8N

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44521 ttgtctgatt tatccaattt atttatctga ctcctgctct ttttttggtt tccatttgca
44581 tggaatattt ttttccatcc cttattttca gtttactgtg tattcttgga caagttactt
44641 tctgagcctc aatttcctca tctgatagtt ggtgtctgtt aggatctatc ttacaggact
44701 gttttaagga ttaaatgaga caatgcacag tgcctggggc atgatgagat atggtaatta
44761 ggaaataatt actcaggcca ggcacggtgg ctcatgccta taattccaac attttgggag
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45061 atagctaaca aatggtagtt attattgtta catttattta tttagaatta ttattcaatt
45121 attattatgt aataatcaac aaaaattatc cttttttgta gcatcttttt atctcttctc
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45421 gaattacaac tcagtgaagg agatatcctc acatgtaact tgcaactcta aatatgacct
45481 tatctaagag ggtattagtt tcaattatgc tgcccctatg ggtcattgaa gtgagactct
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45661 aagacaggga gaaatcacca aatggttttt tttttaaagc aaactaaata tttagccacc
45721 ttttgggttc actttttttt ttctttttct tttttactag tcattgactt gaagctcatg
45781 gttttactct ttaacaccag ccagtcaact gcaggttcct gggacaaaaa gcaaaaggaa
45841 taatgatccc ttgtggattc ctgagtcacc tgctttgtgg gctgtgcagc gtctgtggtc
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46321 tctaaaggcg cttcctgttg cacaagaatg ctgtttgcag gaatgcctgt agacacatga
46381 accctgggcc tgctgctccc ttaatttgag ggaagctatg gatgtcaagc agaatagagc
46441 tattttgtta cttgaccagg tttccataac tttttgcaca tacctttatt cctgcacctg
46501 tcccacataa ttatcccttt atagctcttt caccagcagg aatataaatg tcatggcagg
46561 gccaccatgt tgcaaactcc ctagggtcca tcctgcagtt actagcttgt gtgaatggta
46621 cttcttggag gtaccattgc aatgcacagc ctgagcagcc ctctgcagta agtccaactg
46681 tggatggggc aaaatgagtg gttagcagaa ttggcctcat acatctttta ttctggcatt
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46801 ttttgagtta atttgaccca gcttaatcag tggatctttt caactagtta attgtcctac
46861 ccttccaccc aggaaggtgt ttcagtttta atagattttt gttattggtg ttttttttgca
46921 cattcagcaa aacctctgga aagaggagtt catatcagcc cctcatgcag agggagctgt
46981 gagtattcag gctgcttggt gctgctgcgg tctttccagc agaggtggct ggtcctagag
47041 tggtggtttg gtccattctt gacctatgat gatgctggga aataactcag tgatgtggtg
47101 tcattttttt atctcacaga tgtggctctg gagaagagat ttaagtttat atcttcagaa
47161 catagcatca atgagtttgg gtcatagctg cagccaactc agccagaaac agatacacag
47221 tatgcaacca agtgggaata aattcaaccc attttcctaa tttggaatat agagcctgag
47281 gggtgaagtt gtgggtggat tatctgtcct gggttcttcc tatttcctga atttatactt
47341 tagtgaaggg aaattttgct aactgttaat gtgccagaat gggaaagccc cctacctagg
47401 ccctcacctc tgaattttc cagttcaagt ggacaagtag tttttgagca ctcactatgt
47461 gtcaggctca gggtgacctt tgcgatttcc acttccttct gatcttttca cctaagcctg
47521 tgggacttgt gttctgggag aaaattagaa gttacatcat ccttatggtt ctgaaaaaat
47581 tgagctcccc agacttgggt tcgcctgatc tgcttctcaa atgttaaaga ggaacataaa
47641 gccatgtaga aaatcctttt cttttgacaa agcctggctt tcaaattcta ctgttgctgc
47701 agtcaccaaa attatacatt tgaatgtcag agattgaatg ttgacatcct tattgcaaaa
47761 atcctatccc cacttctggc ttagctgccc cagagagctc tgtgtctcgg gctgactaga
47821 gaaaagacca cacagttaga gatgcaaaaa aggaaatgcc attaacatat ttcagggtga
47881 atatttcttt gcatttggca ctttgcaatc tagtccaggc ttaagatttg taggatttct
47941 tttgctgttt cccttttttt acttgtcagg gaaacaaggc ataaatattt cagcttggac
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19585

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.5, 24.3, 24.31; 530/350, 387.1, 387.9; 435/ 69.1, 71.1, 71.2, 471, 320.1, 254.3, 254.11, 325, 6, 7.1, 7.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,637,470 A (KACZOROWSKI et al.) 10 June 1997 (10.06.97), see entire document.	1-42
A	US 5,776,734 A (KACZOROWSKI et al.) 07 July 1998 (07.07.98), see entire document.	1-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	
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"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search 20 NOVEMBER 2000	Date of mailing of the international search report 27 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Joyce Bridgers for Telephone No. (703) 308-0196

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A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07K 14/47, 16/18; G01N 33/53, 33/567; C12N 5/10, 15/12, 15/63, 15/64

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.1, 23.5, 24.3, 24.31; 530/350, 387.1, 387.9; 435/ 69.1, 71.1, 71.2, 471, 320.1, 254.3, 254.11, 325, 6, 7.1, 7.2

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, CAS ONLINE, MEDLINE, CAPLUS
search terms: calcium sensitive potassium channel beta2, beta3a, beta3b, beta3c, beta3d, nucleic acid, recombinant production, antibody, assay, binding, activator, inhibitor, gene transcription, nuclear factors